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METHYL QUINONES IN OXIDATIVE PHOSPHORYLATION

by

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PREFACE

The work described in this dissertation was carried out in the School of Molecular Sciences, University of Warwick, Coventry between April 1966 and July 1968. It is the original work of the author, except where specific acknowledgement is made, and has not been submitted for a degree at any other University.

The author wishes to thank Professor V. M. Clark, who directed this work, and Dr. D.W. Hutchinson for their constant advice and encouragement.

The award of a Research Studentship by the Science Research Council is gratefully acknowledged.

CONTENTS

ABBREVIATIONS

P_i	inorganic phosphate
ADP	adenosine-5'-diphosphate
ATP	adenosine-5'-triphosphate
NADH	reduced nicotinamide adenine dinucleotide
DMF	N, N-dimethylformamide
k	pseudo first order rate constant
$k_{H(D)/D_2O(H_2O)}$	k for protium (deuterium) compound in deuterium (protium) oxide
TLC	thin layer chromatography
VPC	vapour phase chromatography
NMR	nuclear magnetic resonance
e.s.r.	electron spin resonance
Ad	2',3'-isopropylideneadenosine-5'

SUMMARY

Some evidence for the participation of methyl quinones in oxidative phosphorylation is presented and discussed. Although methyl quinones seem to be definitely involved in electron transport in particulate systems, there is no unequivocal evidence that they are actually involved in the coupling reaction. Several hypothetical schemes for oxidative phosphorylation are discussed with particular emphasis on those involving hydroquinone phosphates. The Vilkas-Lederer scheme and the more recent mechanism proposed by Erickson, Wagner, and Folkers are discussed in detail. An in vitro investigation of the latter proposal is the subject of the present work.

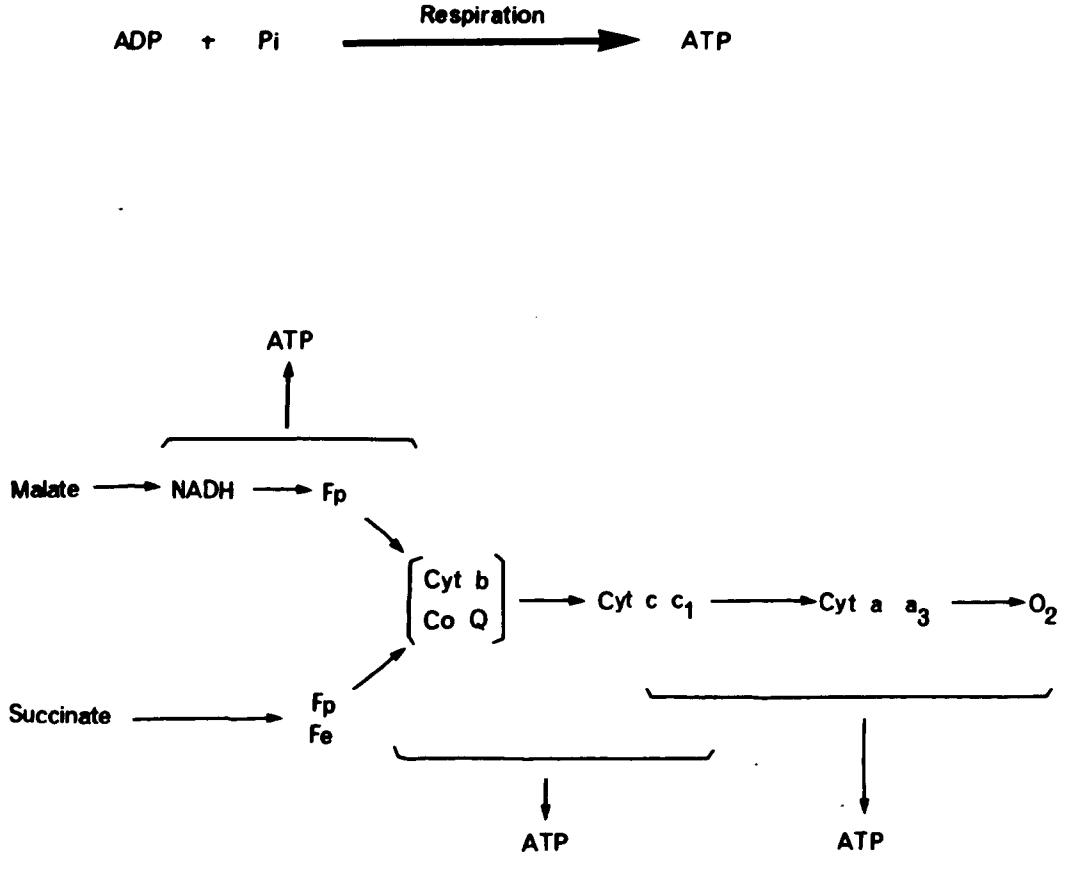
Hydrogen isotope exchange in methyl quinones was observed in the presence of carbonate or triethylamine using the techniques of infra red spectroscopy, NMR spectroscopy, mass spectrometry and scintillation spectrometry. The exchange reactions of duroquinone and 2,3-dimethyl-1,4-naphthoquinone together with their derivatives were investigated over a range of pH and at different temperatures. The large primary isotope effect for the reaction indicates that removal of a proton from the methyl group of the quinone is the rate determining step in those reactions

involving a quinone methide intermediate and offers an explanation for the negative results of the in vivo hydrogen isotope experiments.

The reaction of phosphoric acid and its various anions with both stable and transient quinone methides was investigated. There was no evidence for any nucleophilic addition of a phosphate to a quinone methide, polymerisation of the latter being the only reaction observed.

A synthesis of p-hydroxybenzyl phosphate and a study of its behaviour under oxidising conditions were attempted. Production of p-hydroxybenzyl phosphate in situ under acidic or basic conditions resulted in its immediate decomposition to inorganic phosphate and the quinone methide, precluding any observation of oxidative phosphorylation. No evidence for oxidative acylation with the isolable 2,6-di-t-butyl-4-hydroxybenzyl acetate was obtained.

INTRODUCTION

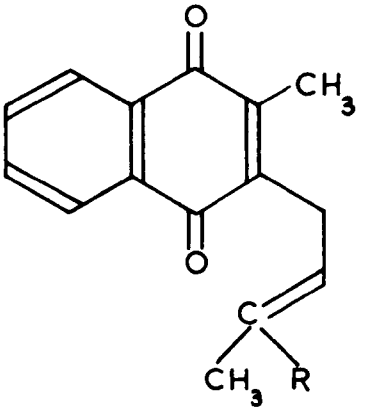


The Respiratory Chain

Figure 1

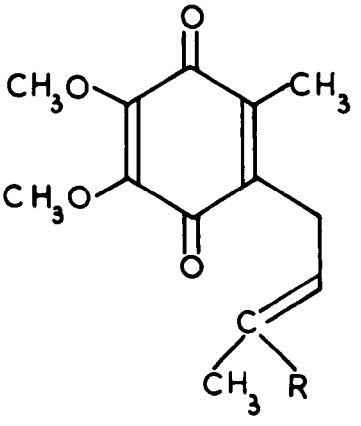
GENERAL INTRODUCTION

Respiring cells derive most of their energy requirement from the oxidation of fatty acids, and various components of the tricarboxylic acid cycle, to carbon dioxide and water. Such oxidation is coupled to the synthesis of ATP from ADP and P_i and much of the free energy released in the oxidation is conserved in the triphosphoryl group of ATP. The overall process is known as oxidative phosphorylation (for reviews see Ref. 1-4) and is catalysed by a complex enzyme system, known as the respiratory chain, which is associated with a membrane structure. In respiring bacteria the enzymes are bound to the cell membrane whilst in most other respiring cells they occur bound to the inner membrane of cytoplasmic organelles known as mitochondria. In mitochondria the transfer of a pair of electrons from a substrate, along the respiratory chain components of increasing redox potential, to molecular oxygen results in the net synthesis of one molecule of ATP from ADP and P_i at each of three respiratory chain coupling sites. The synthesis of ATP and the various components of the respiratory chain are indicated in figure 1. Although the mechanism of oxidative phosphorylation in these systems remains unknown, several schemes have been proposed. An in vitro investigation of one such scheme proposed for the second coupling site is the subject of the present work.

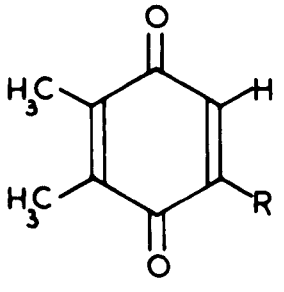


R = $-\left[\mathrm{CH_2-CH_2-CH_2-CH(CH_3)}\right]_3-\mathrm{CH_3}$ I

R = $-\mathrm{CH_2}-\left[\mathrm{CH_2-CH=C(CH_3)-CH_2}\right]_n-\mathrm{H}$ II



R = $-\mathrm{CH_2}-\left[\mathrm{CH_2-CH=C(CH_3)-CH_2}\right]_9-\mathrm{H}$ III



R = $-\left[\mathrm{CH_2-CH=C(CH_3)-CH_2}\right]_9-\mathrm{H}$ IV

Figure 2

EVIDENCE FOR THE INVOLVEMENT OF QUINONES IN ELECTRON TRANSPORT AND PHOSPHORYLATION

Since the naturally occurring Coenzyme Q_{10} (III. figure 2) was first proposed as a component of the respiratory chain in beef heart mitochondria (5), much evidence has accumulated which substantiates this view. It appears that naturally occurring quinones are associated with every type of electron transport system found in animal, plant and microbial cells. The main lines of experimental evidence are :

(a) Extraction Experiments

When quinones are extracted by solvent extraction from systems capable of oxidation phosphorylation, the oxidative and phosphorylative activities are lost and are restored only when the quinone is added to the depleted system. Crane (6) extracted a deoxycholate treated particle from mitochondria with isooctane and found the loss of succinate oxidase activity. The addition of coenzyme Q_{10} and cytochrome c were absolute requirements for the restoration of the activity. Both NADH oxidase and succinate oxidase activity have been restored to acetone extracted mitochondria from the fungus Claviceps by the addition of coenzyme Q (7). The restoration of NADH oxidase activity in beef heart mitochondria has been observed on the addition of coenzyme Q to a pentane extracted preparation (8). Similar extraction experiments have shown that

plastoquinone (IV. figure 2) addition to isooctane extracted chloroplasts restores the Hill Reaction (9), thus implicating plastoquinone in photo-phosphorylation - another system in which electron transport is coupled to the synthesis of ATP and is associated with a membrane structure. A requirement for vitamin K (I and II, figure 2) function in bacteria has been shown by solvent extraction experiments with Mycobacterium phlei (10).

(b) Irradiation Experiments

The endogenous vitamin K in various systems capable of oxidative phosphorylation may be destroyed by irradiation with light of 360 mμ wave-length with resultant loss of oxidative and phosphorylative activity. Addition of vitamin K to the system restores this activity. This technique has shown that vitamin K is involved in electron transport in M.phlei (11) and other bacteria (12, 13). Recently synthetic vitamin K_1 has been resolved into its cis and trans isomers (14). The trans isomer has been found to restore oxidative phosphorylation to irradiated extracts of M.phlei more effectively than the cis isomer. There is no evidence of enzymatic cis-trans isomerisation (15).

(c) Vitamin K deficient cells

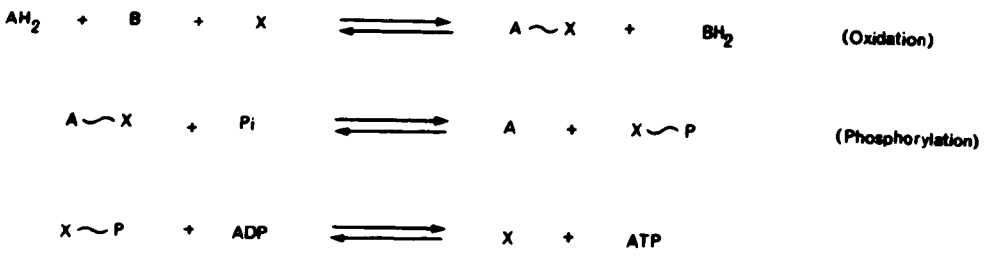
The first suggestion that vitamin K was involved in oxidative phosphorylation came from studies of liver mitochondria from chicks fed on a vitamin K deficient diet (16). A low P:O ration was observed

and addition of 10^{-5} M vitamin K stimulated phosphorylation coupled to oxidation. Certain mutants of Escherichia coli deficient in Coenzyme Q are found to have a low NADH oxidase and malate oxidase activity which can be restored to that of the wild type by the addition of coenzyme Q (17, 18).

(d) Oxidation-reduction of endogenous quinones

Extraction techniques followed by optical determination of the relative amounts of the quinone in the oxidised and reduced forms have shown that quinones undergo oxidation and reduction both in whole mitochondria and in mitochondrial particles (19, 20, 21). Under anaerobic conditions and in the presence of substrate there is a build up of the hydroquinone form, while under aerobic conditions this is reoxidised to the quinone (22, 23). Kinetic experiments, using a double beam spectrophotometric technique on whole mitochondria to compare the rates of the redox reactions of coenzyme Q with those of other carriers and the overall rate of electron transport, suggested that coenzyme Q and cytochrome b may be alternative pathways in the chain (24, 25). However it could be that a small part of the quinone undergoes rapid oxidation-reduction to account for the main electron transport.

Scheme (i)



Scheme (ii)

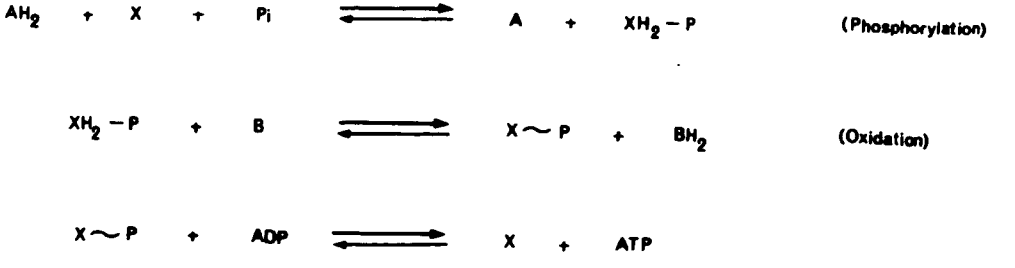


Figure 3

THEORIES OF OXIDATIVE PHOSPHORYLATION

General Schemes

Two general mechanisms of oxidative phosphorylation based on biochemical principles may be formulated (26) as shown in figure 3. The first scheme (27) is an analogous scheme to that proposed (and later substantiated) for the oxidative phosphorylation associated with glyceraldehyde-3-phosphate dehydrogenase (28). The energy of electron transport from carrier A to B is transferred to the intermediate A~X containing a high energy bond. The carrier, A, is regenerated when the intermediate, A~X, is phosphorylated to form X~P; X itself is regenerated when the intermediate, X~P, phosphorylates ADP to form ATP. The second scheme (29) reverses the order of the oxidative and phosphorylative steps. Evidence for such stepwise schemes is provided, in part, by the action of various inhibitors. In particular dinitrophenol uncouples oxidative phosphorylation (30) - that is, respiration is no longer coupled to phosphorylation. Oligomycin inhibits oxidative phosphorylation (31) and furthermore this inhibition is completely released by dinitrophenol (32), indicating that the two compounds act at different points in the sequence of reactions. Several of the partial reactions predicted by the above schemes have been observed. An ATP-$^{32}P_i$ exchange has been observed in liver

mitochondria (33, 34). An ADP - ATP exchange has been observed (35) and in intact mitochondria has been shown to be both oligomycin and dinitrophenol sensitive (36). The probable reason for the latter result is that using intact mitochondria one can maintain oxidative phosphorylation without the addition of magnesium ions. In particulate fractions the addition of magnesium ions is required and this also stimulates adenylate kinase which can also give rise to an ATP - ADP exchange. An H_2O^{18} - P_i exchange has been observed and is sensitive to dinitrophenol (37).

Hypothetical schemes involving the oxidation of a hydroquinone phosphate yield ATP in a scheme of the second type. However present biochemical evidence tends to favour a scheme of the first type. Submitochondrial particles, unlike intact mitochondria, contain only trace quantities of adenine nucleotides and inorganic phosphate. Although these particles cannot use the energy of respiration to generate ATP unless both ADP and P_i are added, they may use the energy for endergonic reductions such as the transhydrogenase reaction (38, 39). Also the succinate linked energy dependent reduction of acetoacetate (40) and α-ketoglutarate (41) can proceed in the presence of oligomycin which blocks the synthesis of ATP. This evidence suggests the participation of a high energy intermediate not containing phosphate and thus favours a scheme of the first type.

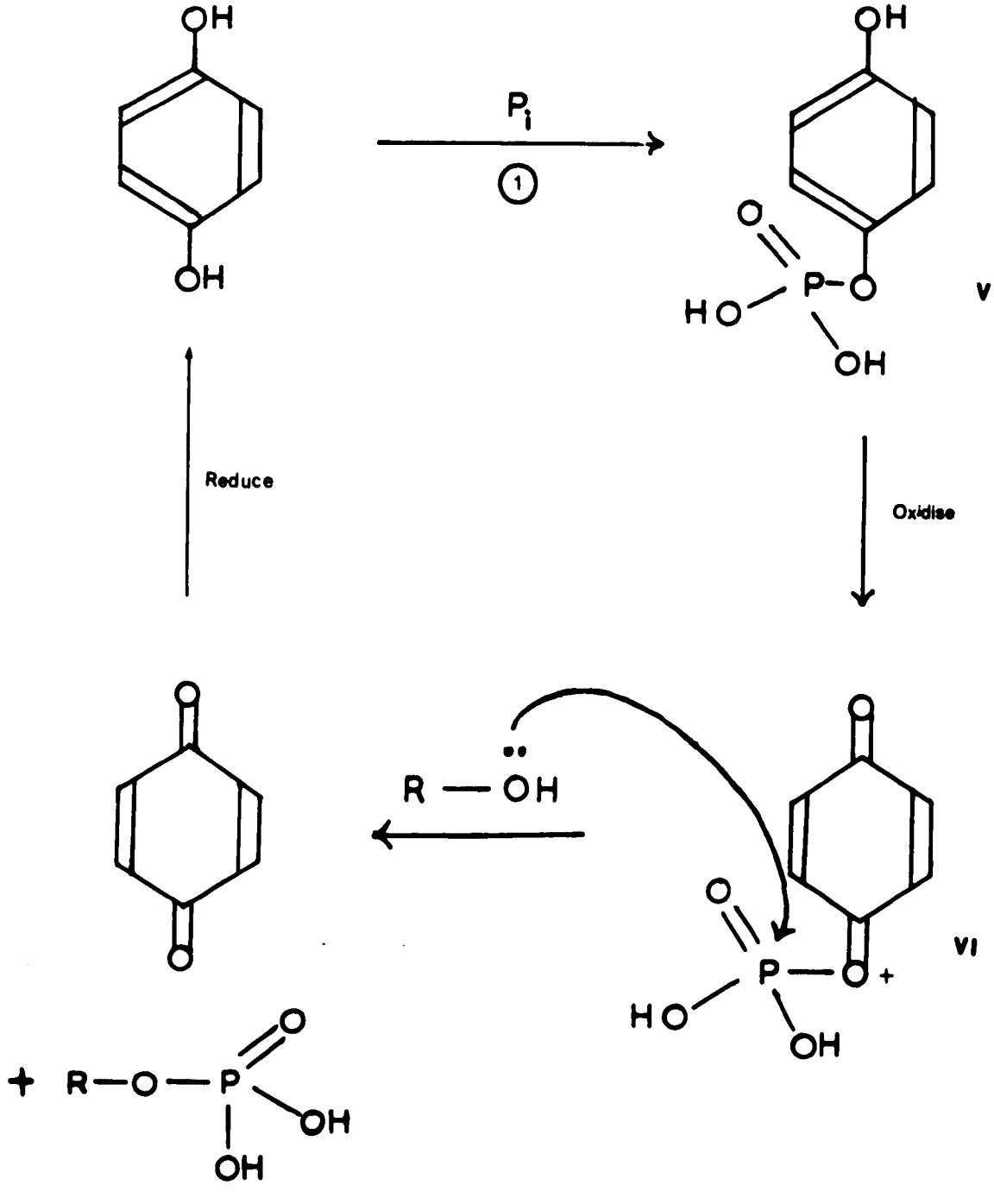


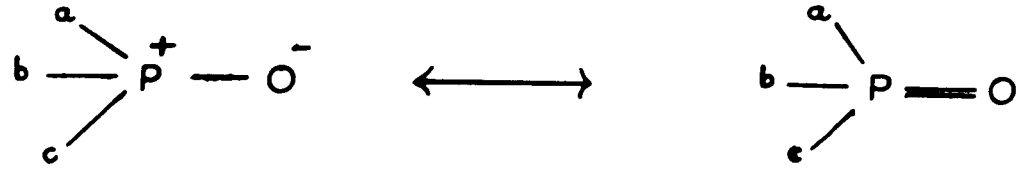
Figure 4

SCHEMES INVOLVING HYDROQUINONE PHOSPHATES

In support of a scheme of the second type, organic chemists have suggested several possible mechanisms. Wessels (42) was the first to suggest a scheme using a hydroquinone phosphate to explain the role of quinones in oxidative phosphorylation. Many similar schemes followed (43 - 50). The essential features of the schemes are indicated in figure 4. The oxidation of a hydroquinone phosphate (V) yields the intermediate (VI) which can react with a nucleophile by attack at the phosphorus atom to yield quinone and a phosphorylated nucleophile.

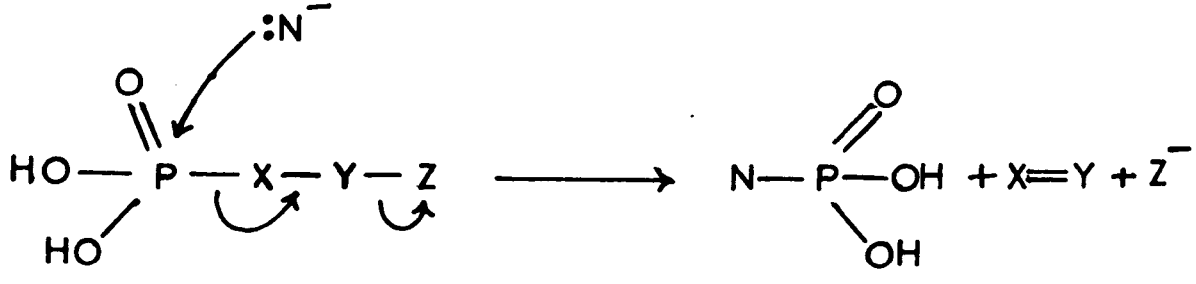
(a) Hydroquinone phosphates and their oxidation

The attack by a nucleophile on the phosphorus atom of the P = 0 bond mentioned above is difficult to analyse because the atom is tetrahedrally surrounded and thus sterically hindered. Furthermore the nature of the π-bonding is not completely understood. The phosphorus atom in a phosphoryl group is sp^3 hybridised and may be considered as bearing a formal positive charge. This results in a contraction of the d-orbitals of the phosphorus atom, so that they become of comparable energy to the oxygen p-orbitals and can overlap with them. The oxygen can donate a pair of electrons to the phosphorus atom and



pπ – dπ bonding

(a)



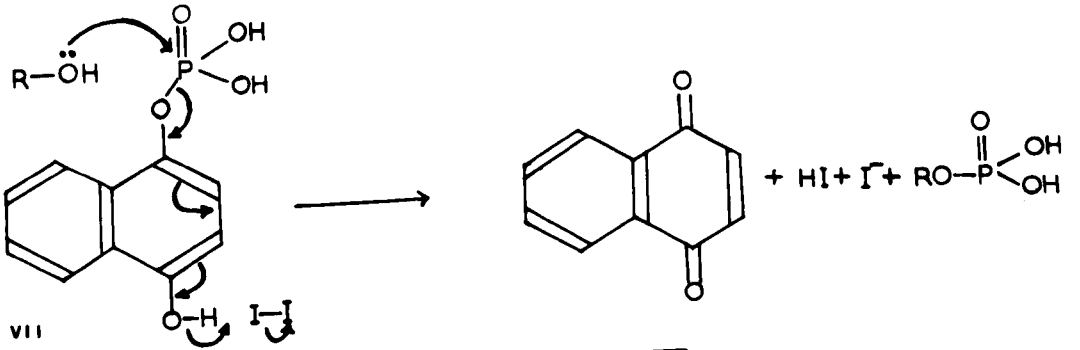
The P–XYZ System

(b)

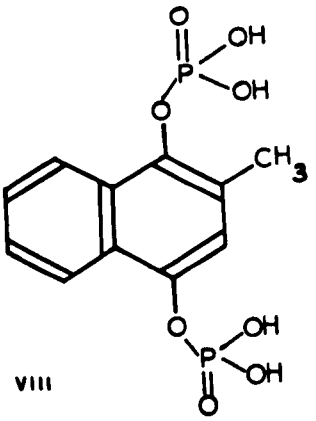
Figure 5

thus the P = 0 bond has some $d\pi$ - $p\pi$ character and the bond is thereby strengthened (figure 5(a). Ref. 51). A hydroquinone phosphate is essentially a vinylogous perphosphate and is a vinylogous P-XYZ system (52). Such a system is a phosphorylating agent if the electrons of the P-X bond can be formally accommodated on Z (figure 5(b)). In a phosphorylation reaction the bond order between X and Y must increase by one unit and that between Y and Z must decrease by one unit. Z is the electron acceptor and so must be highly electronegative or become so under oxidising conditions or attack by an electrophile. The P-X bond should be relatively weak if the P-XYZ system is to be a good phosphorylating agent. The $d\pi$ - $p\pi$ overlap between P and X may be reduced by the introduction of pure $p\pi$ bonding with an sp^2 hybridised Y atom and is zero when X is an sp^3 hybridised carbon atom. In the case of a hydroquinone phosphate the oxygen p electrons are to some extent accommodated on the phenyl ring and thus the $d\pi$ - $p\pi$ overlap between P and X is attenuated. Perphosphoric acid (X = Y = 0, Z = H, Ref. 53) becomes a phosphorylating agent under oxidising conditions. A similar oxidation of naphthohydroquinone phosphate (VII. figure 6a) provided the first experimental evidence for those schemes of oxidative phosphorylation which involved the oxidation of a hydroquinone phosphate in the phosphorylating step (54).

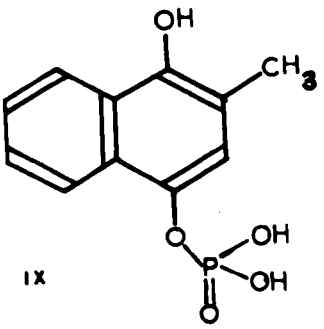
Figure 6 (a)



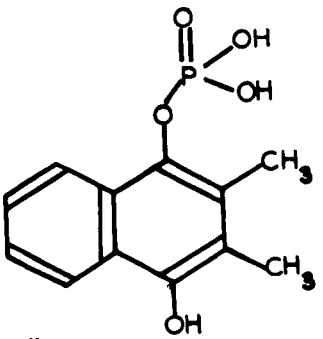
VII



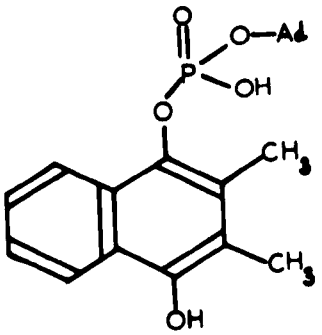
VIII



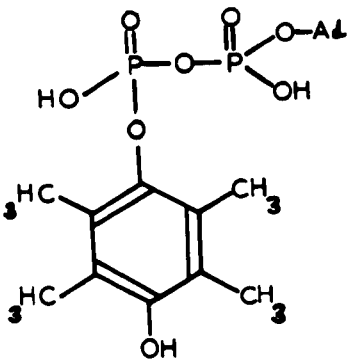
IX



X



XI



XII

Figure 6 (b)

Further information on similar reactions soon accumulated. The oxidation of Synkavit (VIII, figure 6b) with bromine in aqueous solution to the corresponding quinone (55, 56) gave 10% inorganic pyrophosphate while in non-aqueous solution (N, N-dimethylformamide) it gave 15% pyrophosphate and 35% trimetaphosphate. Similarly in non-aqueous solution the bromine oxidation of 1-hydroxy-2-methyl-naphthyl-4-phosphate (IX, figure 6b) gave inorganic pyrophosphate in the presence of added inorganic phosphate (57, 58). The bromine oxidation of 2, 3-dimethyl-4-hydroxy-naphthyl-1-phosphate (X, figure 6b) in dry N, N-dimethylformamide in the presence of the mono-tetrabutylammonium salt of AMP led to the formation of ADP in 26% yield. No ATP was detected in the reaction mixture by paper chromatography or paper electrophoresis. Similarly bromine oxidation of the naphthohydroquinone ester of protected AMP (XI, figure 6b) in the presence of tetrabutylammonium dihydrogen phosphate in dry N, N-dimethylformamide led to ADP in 22% yield. The oxidation of the corresponding durohydroquinone ester of protected ADP (XII, figure 6b) under similar conditions gave ATP in 13% yield (59). However such a process is unlikely to occur <u>in vivo</u> for $^{18}0$ studies have revealed that the ADP in fact furnishes the terminal bridge oxygen ot ATP (60). The mechanism of oxidative phosphorylation reactions with hydroquinone phosphates appears to be quite complex. While

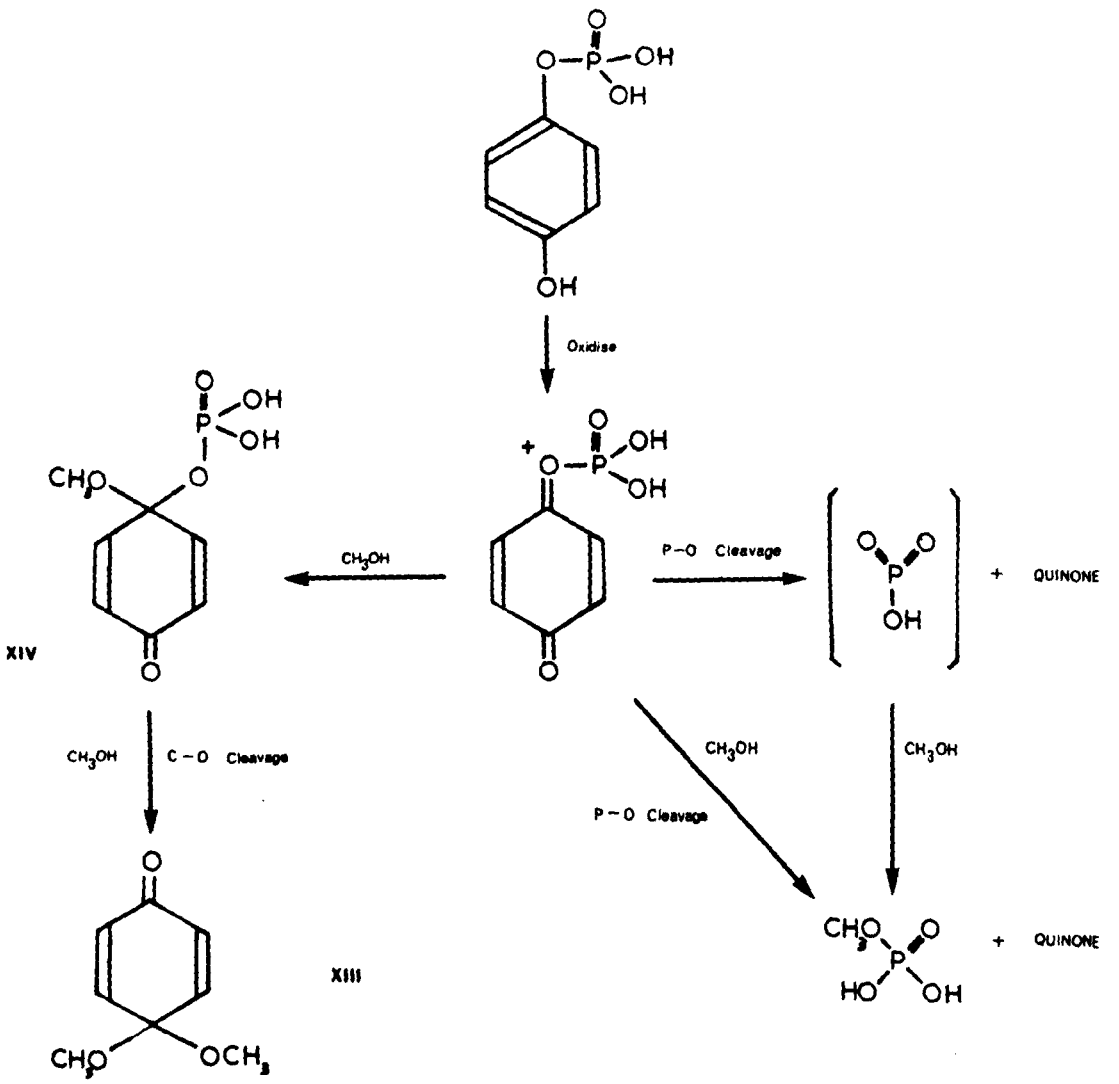

Oxidise
CH3OH
P–O Cleavage
+ QUINONE
XIV
CH3OH
C – O Cleavage
CH3OH
P – O Cleavage
CH3OH
XIII
+ QUINONE

Figure 7

Wieland (61) favours nucleophilic attack at phosphorus, Todd (62) favours the initial expulsion of monomeric metaphosphate. The formation of polyphosphates in non-aqueous solvents and the fact that hydroquinone phosphate diesters (which cannot yield metaphosphate without hydrolysis) do not break down even in the presence of ceric sulphate favour this latter view. Dallam (63) observed that the oxidation of 4-hydroxy-3 methyl-1-naphthyl phosphate by bromine in the presence of $^{32}P_i$ yielded no pyrophosphate, but his suggestion that this observation excluded metaphosphate as an intermediate is invalid as the oxidation was carried out in aqueous solution and the water, being in large excess with respect to the $^{32}P_i$, would rapidly solvate any metaphosphate to form inorganic phosphate. Isotopic studies with $^{18}0$ have shown that in dry N, N-dimethylformamide the bromine oxidation of 2, 3-dimethyl 4-hydroxy-1-naphthyl phosphate in the presence of $[^{18}0]$ P_i yields pyrophosphate, whose $^{18}0$ content together with that of the product P_i, indicated 30% P-0 bond fission (64). Similar results were obtained in aqueous solution (65). The oxidation of benzohydroquinone phosphate in methanol gives the ketal of benzoquinone (XIII, figure 7) in 80 - 90% yield as well as the benzoquinone in 10 - 20% yield (66). A possible mechanism for the phosphoryl transfer explaining these results is indicated in figure 7. Phosphorus-oxygen cleavage can arise by nucleophilic attack of methanol at phosphorus or by the elimination of monomeric metaphosphate. Carbon-oxygen cleavage can occur by attack of methanol on the semi-ketal phosphate (XIV, figure 7).

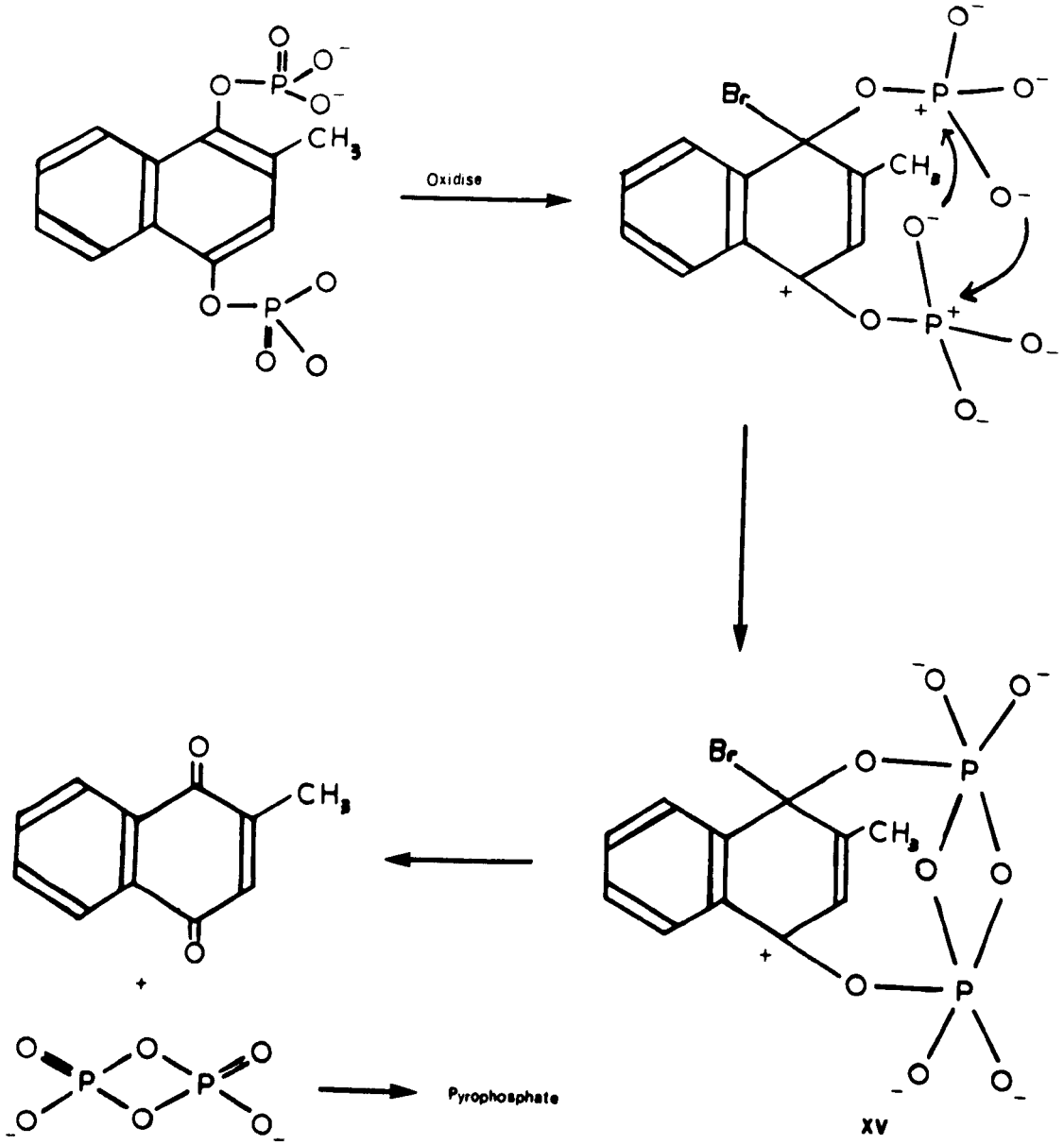


Figure 8

In the case of Synkavit (VIII, figure 6) some pyrophosphate is formed when the oxidation is carried out in aqueous solution, but in the presence of $^{32}P_i$ no labelled pyrophosphate is produced (67, 68). An intramolecular reaction mechanism was suggested to explain these results and further substantiated by the observations that the bromine oxidation of 4-acetoxy-3-methyl-1-naphthyl phosphate led to the formation of acetyl phosphate and inorganic phosphate. Using $^{32}P_i$ in the system, no labelled acetyl phosphate was produced (63). The suggested mechanism is indicated in figure 8. On oxidation the two phosphate groups come into close proximity and can form the bridged intermediate indicated (XV, figure 8). However when the oxidation of Synkavit is carried out in DMF in the presence of $^{32}P_i$, labelled pyrophosphate and polyphosphates are formed. Ethanol added to the reaction mixture before the oxidation forms labelled ethyl phosphate, although the ethyl phosphate is unlabelled in the absence of DMF. This suggests that DMF participates in the reaction and first solvates any eliminated metaphosphate. No yields are quoted in the above work and one would suggest that both intramolecular reactions and elimination of metaphosphate occur in both the aqueous and non-aqueous reactions. The participation of N, N-dimethylformamide in this reaction was confirmed using [^{18}O] DMF as solvent, when the bromine oxidation of Synkavit

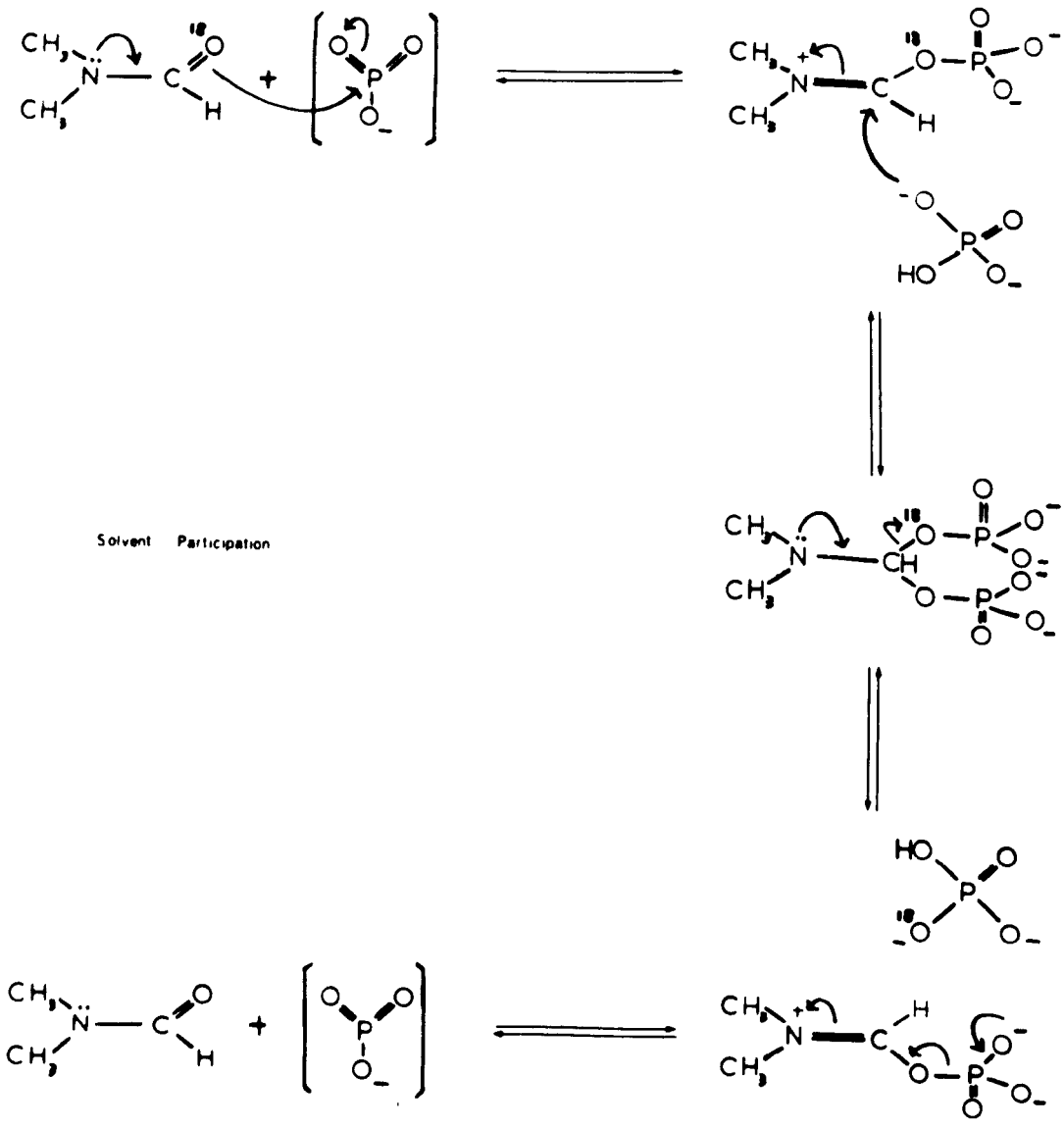


Figure 9

produced ^{18}O-labelled inorganic phosphate consistent with 26% phosphorus-oxygen cleavage. The suggested mechanism for the exchange is shown in figure 9. The [^{18}O]DMF solvates any eliminated metaphosphate to form an imidoyl phosphate which can be attacked by inorganic phosphate either at phosphorus, with formation of pyrophosphate and elimination of [^{18}O]DMF, or at carbon with formation of the symmetrical intermediate shown in the diagram. This can eliminate an unlabelled or an ^{18}O-labelled molecule of inorganic phosphate to form the imidoyl phosphate again. Imidoyl phosphates are well known phosphorylating agents and further examples are those formed from inorganic phosphate and carbodiimides (70), trichloracetonitrile (71), and isocyanates (72).

The extent of phosphorus-oxygen cleavage during the oxidation of a hydroquinone phosphate may well be under enzymatic control. Under the action of horseradish peroxidase durohydroquinone phosphate is oxidised by hydrogen peroxide, and ^{18}O studies have revealed that there is 91% phosphorus-oxygen cleavage (73). Binding of the durohydroquinone phosphate to the active site of the enzyme could render the molecule able to cleave at one position only. When a molecule is bound to an active site of a macromolecule its environment may be completely different to its environment in the bulk solution.

(b) The Problem of formation of a hydroquinone phosphate from a hydroquinone or quinone

A problem with the early schemes for oxidative phosphorylation involving a hydroquinone phosphate was the formation of a hydroquinone phosphate from a hydroquinone (e.g. reaction 1, figure 4). Such a reaction is endergonic and any net gain in energy from the formation of ATP would be nullified. 1, 2-Addition of inorganic phosphate to one of the carbonyl groups of the quinone followed by a reduction of the resulting adduct to a hydroquinone phosphate has been suggested (50). A development of this mechanism (74) involved the attack of inorganic phosphate on the 4-carbon of vitamin K_1 with simultaneous cyclisation of the molecule to a chromane. Reduction of the adduct resulted in formation of a hydroquinone phosphate substituted with a hydroxyl group in the γ-position of the side chain. This compound was dehydrated to form the hydroquinone phosphate of vitamin K_1. However both these schemes involve cleavage of the carbon-oxygen bond of the quinone and would thus imply that the quinone oxygen would exchange. ^{18}O studies in vivo have not detected such an exchange (75).

Schemes involving the addition of inorganic phosphate to a quinone methide (76) were the next development in the proposed mechanisms for oxidative phosphorylation. In the first such scheme, formation of the quinone methide with simultaneous cyclisation to a furan ring

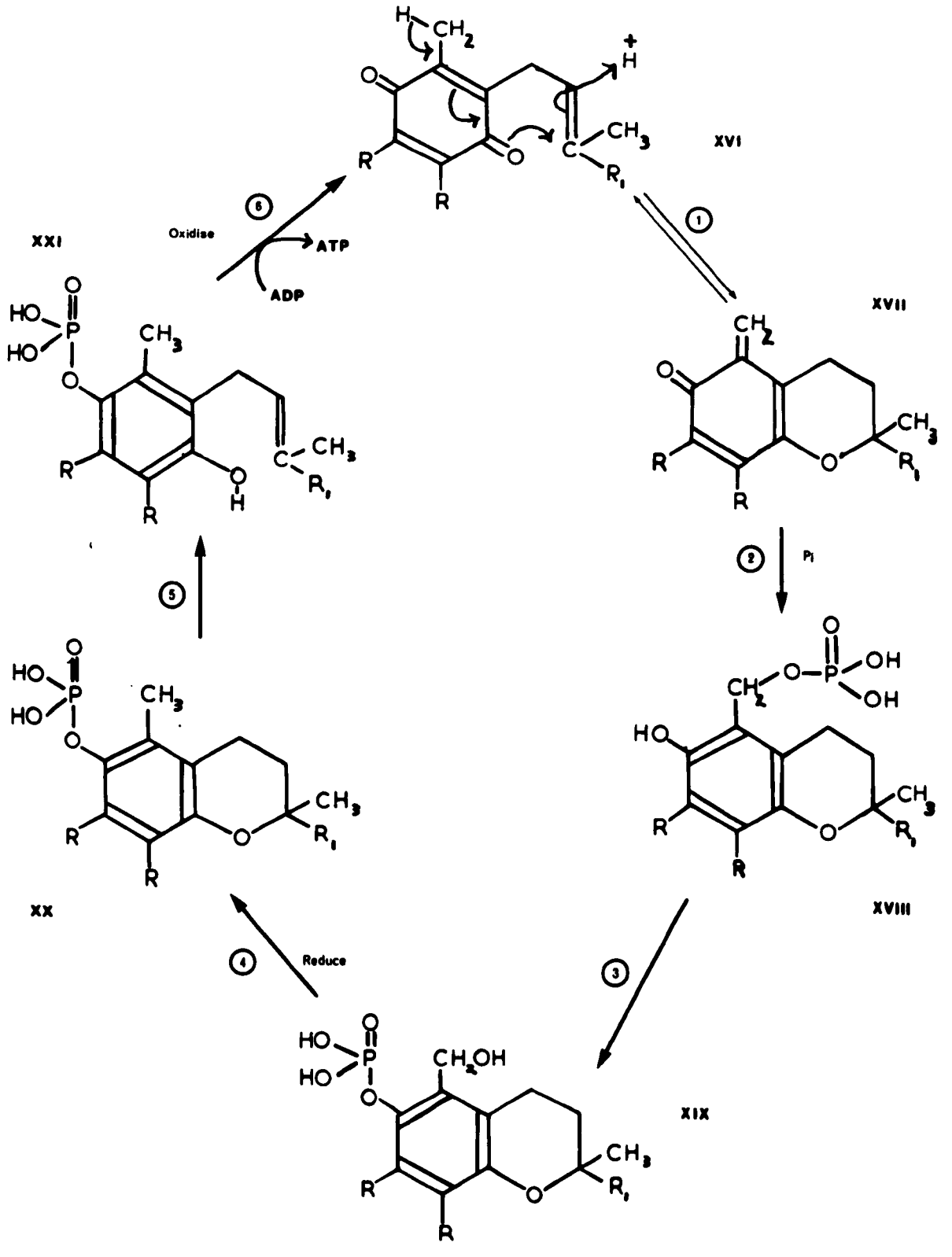


Figure 10

THE VILKAS – LEDERER SCHEME

involving the side chain of the quinone, was followed by a 1,2-addition of inorganic phosphate to the carbonyl group of the quinone methide (77). However this scheme again implied an exchange of quinone oxygen which has not been observed (75). The most sophisticated scheme involving a quinol phosphate is that proposed by Vilkas and Lederer (78) and illustrated in figure 10. A 1,4-addition of inorganic phosphate to the quinone methide is suggested in this scheme which will now be considered in detail.

THE VILKAS-LEDERER SCHEME (figure 10, Ref. 78)

(a) Biochemical Evidence

The Vilkas-Lederer scheme takes account of the following biochemical observations.

(i) The quinone must have both a ring methyl group (79) and a β,γ-unsaturated side chain (80) in order to function in oxidative phosphorylation.

(ii) Inorganic phosphate labelled with ^{18}O exchanges its label with water (33).

(iii) ADP provides the terminal bridge oxygen in ATP (60).

(iv) No exchange of quinone oxygen occurs (75).

The scheme consists of the following steps (see figure 10). Cyclisation of the quinone, XVI, leads to the quinone methide, XVII,

which reacts with inorganic phosphate in a 1,4-addition reaction to give the aralkyl phosphate, XVIII. Migration of the phosphoryl group from the benzylic oxygen to the phenolic oxygen atom produces the chromanyl phosphate, XIX. Reduction of the benzyl alcohol moiety yields the chromanyl phosphate, XX, which is considered to be an intermediate in oxidative phosphorylation. This chromanyl phosphate, XX, isomerises to the hydroquinone phosphate, XXI, which is then oxidised to give the quinone and phosphorylates ADP to form ATP. Each stage of the reaction will now be considered in more detail.

(b) The isomerisation of a quinone to a quinone methide

The isomerisation of a quinone to a quinone methide is enhanced by the simultaneous cyclisation of the β,γ-unsaturated side chain to a chromane. To isomerise 2,3-dimethyl-1,4-naphthoquinone to the quinone methide requires 20.3 kcals./mole, whereas the quinone, XVI (figure 10), and the quinone methide, XVII (figure 10), have almost equal energies (2). The proposed equilibrium between the quinone and the quinone methide implies that hydrogen isotope exchange in the methyl group of the quinone should occur. Although the observation of such exchange has been attempted by several workers, none have observed it. In vivo experiments using vitamin K

(81, 82) and coenzyme Q (83) in which the ring methyl group was labelled with both ^{14}C and ^{3}H showed no alteration in the $^{14}C:^{3}H$ ratio when the quinone was added to rat liver mitochondria or cell free preparations of M.phlei undergoing oxidative phosphorylation. The positive incorporation of tritium into endogenous vitamin K_2 or added vitamin K_1 has been observed during oxidative phosphorylation with cell free extracts of M.phlei in tritiated water (84). However other workers have been unable to repeat these results (85, 86, 82). There is no evidence of carbon-hydrogen bond cleavage in quinones during oxidative phosphorylation (86). Similarly no isotope exchange has been observed in any in vitro experiments. Because of its simplicity, for it contains but one type of carbon-hydrogen bond, many experiments have been carried out with duroquinone. The NMR spectrum of duroquinone in deuteromethanol (MeOD) and sodium methoxide showed a reduction in the peak height of the methyl signal with time. This was interpreted as evidence for deuterium exchange (87) but this interpretation was shown to be incorrect as peak broadening rather than a change in the area under the peak occurred, and acidification of the solution restored the peak to its original height (88). The peak broadening was later shown by e.s.r. spectroscopy to be due to the formation of

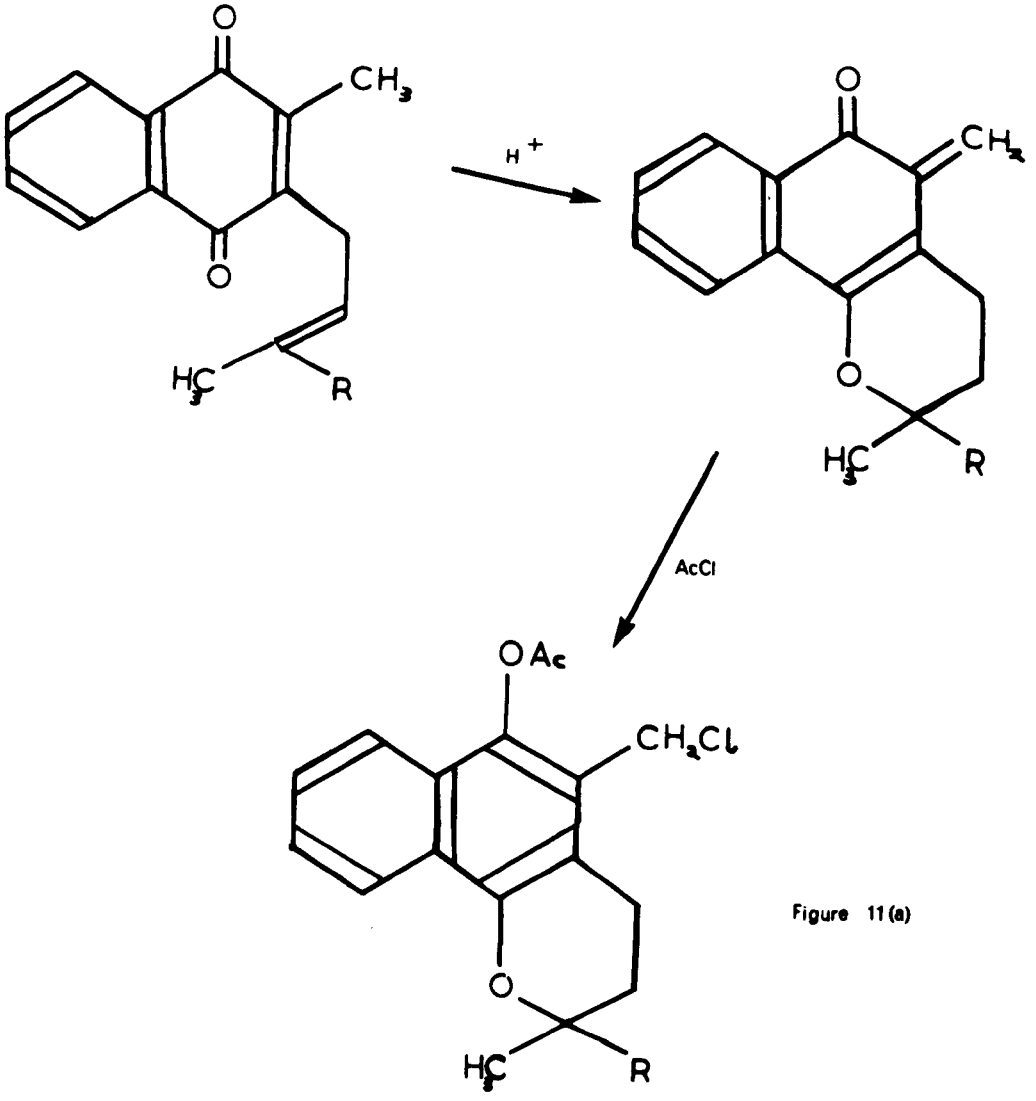
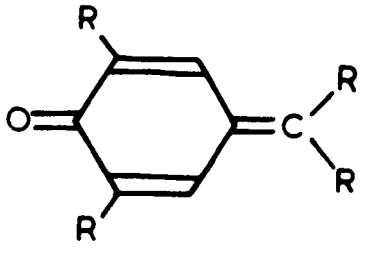


Figure 11(a)

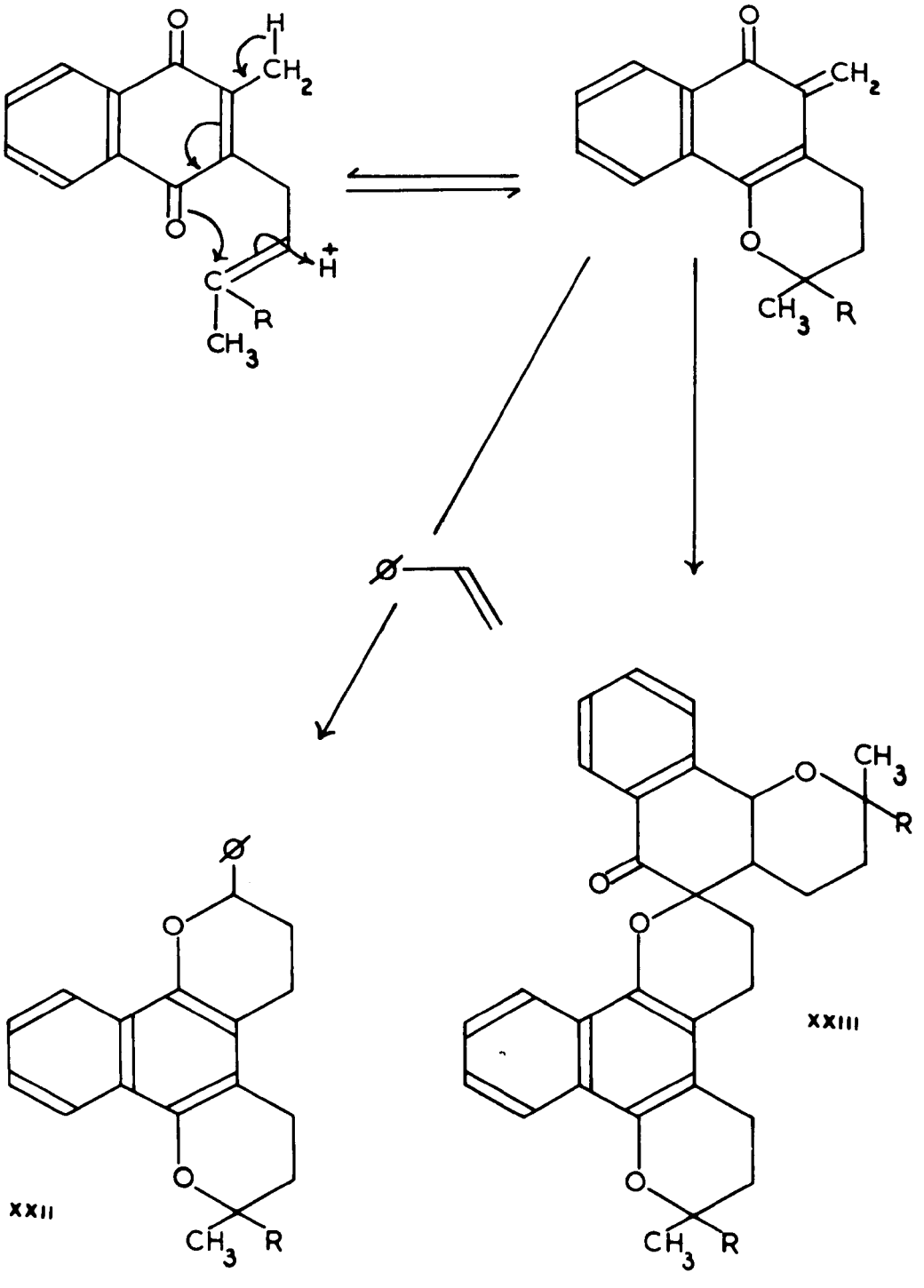


XXIV

Figure 11(b)

radicals among which is the durosemiquinone radical anion, identified by the hyperfine structure of the spectrum (89). Solutions of 2,3-dimethyl-1,4-naphthoquinone in dioxan/deuterium oxide from apparent pH1 to apparent pH 9 were heated under reflux for several days but in each case the recovered 2,3-dimethyl-1,4-naphthoquinone showed no deuterium incorporation (89). It appears there is no evidence for the equilibrium between a methyl quinone and a quinone methide either from the in vivo or the in vitro experiments.

Several reactions have been observed which appear to proceed via a quinone methide intermediate. Methyl quinones undergo side chain amination on treatment with a secondary amine (90). The reaction is indicated in figure 15. Formation of the quinone methide, XXXIV, from the quinone is followed by a nucleophilic 1,4-addition reaction of the secondary amine with the quinone methide, XXXIV, to form the monosubstituted hydroquinone, XXXV, which then undergoes oxidation to the quinone followed by a further similar addition of the secondary amine to form the disubstituted hydroquinone, XXXVI, as the final product. The reaction of vitamin K_1 with acetyl chloride in perchloric acid solution is considered to involve a quinone methide intermediate (91). The reaction is indicated in figure 11(a). Vitamin K_1 isomerises to the quinone methide, under the acidic conditions,



XXII

XXIII

Figure 12

with simultaneous cyclisation of β,γ-unsaturated side chain to a chromane. Acetyl chloride then reacts with the quinone methide in a 1, 4-addition reaction. Similarly the dimerisation reaction of vitamin K_1 in perchloric acid/acetic acid solution to produce the dimer, XXIII (figure 12), is considered to involve initial formation of a quinone methide intermediate (92). Furthermore the quinone methide may be trapped using styrene to give the adduct, XXII (figure 12, Ref. 93). The structures of the compounds were determined spectroscopically. In an acid medium the presence of an unsaturated side chain appears to be essential for quinone methide formation because neither styrene adduct formation nor dimer formation can be detected under similar conditions using 2', 3'-dihydrophylloquinone in place of vitamin K_1(2). Furthermore duroquinone, which contains no unsaturated side chain, forms diduroquinone under alkaline conditions (94).

(c) The addition of phosphate to a quinone methide

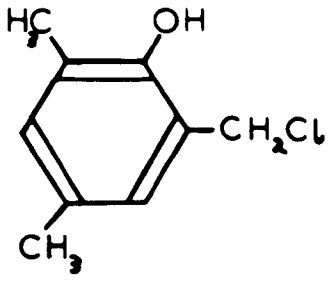
The second reaction in the Vilkas-Lederer scheme (reaction 2, figure 10) involves the 1, 4-addition of inorganic phosphate to a quinone methide.

Quinone methides (general formula, XXIV, figure 11(b)) are related to the quinones by the replacement of one oxygen by a methylene group. All synthetic and natural stable quinone methides, except for

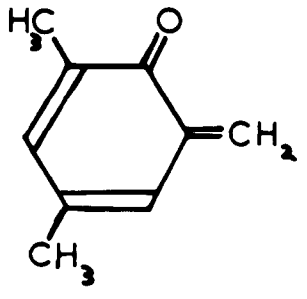
9, 10-anthraquinone methide (95), have a substituted methylene group. As discussed earlier the less stable quinone methides have been suggested as transient intermediates in chemical reactions, both in vitro and in vivo. Quinone methides and their properties have been reviewed in the literature (96, 97, 98). Their high reactivity is greater than that observed with quinones and results from their dipolar character and the energy obtained from aromatisation of the ring, leading in particular to nucleophilic addition and polymerisation at the methylene carbon atom. Nucleophilic addition to quinone methides has been observed in both acid and basic solution with both stable quinone methides (99, 100, 101) and transient quinone methide intermediates (102, 103, 104, 105). Para-quinone methides often disproportionate to give equal quantities of stilbene-quinones and dihydroxy-biphenylethanes (106). The formation of such compounds is often taken as evidence of transient quinone methide formation when the latter cannot be isolated.

In the literature there are only two claims to have observed the direct addition of phosphoric acid to a quinone methide. In an American Patent (107), Thompson claims to have added both mono-

XXV



XXVI



XXVII

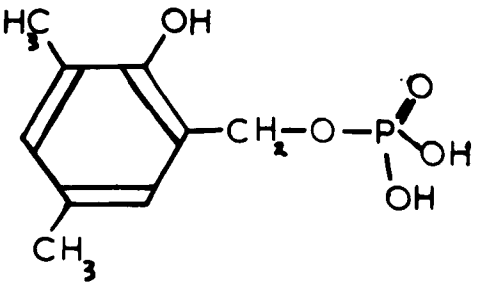


Figure 13

and dioctyl orthophosphoric acid to 2,6-di-t-butyl-4-isopropylidene-2,5-cyclohexadiene-1-one in benzene saturated with gaseous anhydrous hydrogen chloride. However no data about the product is given. Vilkas (108) claims that the chloromethyl phenol, XXV (figure 13), on treatment with an equimolar mixture of orthophosphoric acid and tributylammonium dihydrogen phosphate in acetonitrile,yields the hydroxybenzyl phosphate ester, XXVII (figure 13), via a quinone methide intermediate, XXVI (figure 13). The formation of the phosphate ester does not result from a direct nucleophilic substitution of the chlorine atom by phosphate as the 0-methyl ether of XXV does not react under similar conditions. Also there is a transient yellow coloration which appears on mixing the two reactants and is characteristic of a quinone methide. However no details of the product are given and the paper claimed to be in preparation has not been published after two years. There is no unequivocal evidence for the addition of phosphate to a quinone methide.

(d) Other reactions in the Vilkas-Lederer Scheme

The third reaction in the Vilkas-Lederer scheme (reaction 3, figure 10) involves the migration of a phosphoryl group from a benzylic oxygen to a phenolic oxygen atom. This transesterification step is thermodynamically unfavourable for it requires a phenol

(a stronger acid) to displace an alcohol (a weaker acid) and has not been observed in a chemical system.

The fourth reaction of the scheme (reaction 4, figure 10) involves the reduction of a benzyl alcohol to the hydrocarbon and reactions of this type are well known (109).

Isomerisation of the chromanyl phosphate, XX, to the hydroquinone phosphate, XXI, (reaction 5, figure 10) is endergonic and is therefore unlikely to occur. The NMR spectrum of 2,2,5,7,8-pentamethyl-6-chromanyl phosphate, even on ultraviolet irradiation, shows that there is no exchange of deuterium with alkaline deuterium oxide or acidic deuteromethanol (87). Similarly the free chromanol shows no deuterium isotope exchange in either acidic or alkaline deuteromethanol. Of course such negative results cannot be conclusive evidence that such an exchange does not occur.

The reaction completing the scheme (reaction 6, figure 10) involves the oxidation of the hydroquinone phosphate, XXI, with resultant formation of the quinone, XVI, and phosphorylation of ADP to form ATP. The oxidation of hydroquinone phosphates has been discussed in detail previously.

(e) Chromanyl Phosphates as intermediates in oxidative phosphorylation

The chromanyl phosphate, XX (figure 10), is considered to be an intermediate in oxidative phosphorylation (2) and considerable

effort has been made in an attempt to demonstrate this.

Incubation of vitamin K_1 and inorganic phosphate under anaerobic conditions with extracts of M.phlei leads to the formation of a phosphorylated reduced derivative of vitamin K_1 in 0.3 - 0.7% yield (110, 80). This was shown chromatographically using tritium-labelled vitamin K_1 and $^{32}P_i$. This enzymically formed reduced phosphorylated derivative is oxidised in the air to vitamin K_1 with release of inorganic phosphate. It was suggested that the derivative may be the 6-chromanyl phosphate of vitamin K_1, but its spectroscopic and chromatographic properties are slightly different from synthetic 6-chromanyl phosphate of vitamin K_1 (111). The phosphorylated derivative served as an electron donor when incubated with fresh extract and ADP under aerobic conditions and ATP was produced (112). ATP was not produced under anaerobic conditions suggesting that oxidation was necessary for ATP formation. Using ^{32}P-labelled intermediate under aerobic conditions, ^{32}P-labelled ATP was produced. However there is no evidence that there is direct phosphoryl transfer from the intermediate to ADP. This could have been investigated by adding unlabelled inorganic phosphate to the system before

incubation and examining the extent of the ^{32}P-labelling of the ATP produced. The extracts of M.phlei contain little endogenous inorganic phosphate and it is possible that an oxidative dephosphorylation of the intermediate occurs with oxidative phosphorylation then occurring in the usual way using the liberated inorganic phosphate. The extract of M.phlei contains adenylate kinase (113), which catalyses the following equilibrium :

$$ADP + P_i \rightleftharpoons ATP$$

This can lead to the formation of ATP by a route not involving oxidative phosphorylation. No correction for such activity is mentioned in the above experiments. Synthetic 6-chromanyl phosphate of vitamin K_1 has been studied in a cell-free preparation of M.phlei capable of oxidative phosphorylation (113, 114). The system consisted of the synthetic phosphate, M.phlei extract, cytochrome c,which functioned as an electron acceptor, and a phosphate acceptor system consisting of glucose, hexokinase and ADP. This system oxidised the synthetic 6-chromanyl phosphate and glucose 6-phosphate was produced, indicating the formation of ATP. However the corrections due to ATP formation by inherent adenylate kinase activity were of the order of five times (117)

the ATP produced by the oxidation of the 6-chromanyl phosphate, hence it is doubtful if the results are meaningful.

Wieland (115) claims to have prepared the hydroquinone 1-phosphate of coenzyme Q6 by the method of Andrew's (116), but it has been shown that the debenzylation step in this synthesis, for example by a Lindlar catalyst, also results in extensive reduction of the double bonds in the isoprenoid side chain and hence the authenticity of Wieland's compound is in doubt. The presumed hydroquinone 1-phosphate of coenzyme Q6 was oxidised slowly by mitochondria with formation of ATP. The ATP produced was used in a phosphate acceptor system consisting of glucose and hexokinase. Using substrate labelled with $^{32}P_i$, glucose 6-phosphate labelled with $^{32}P_i$ was obtained and dilution of its ^{32}P-content did not occur if $^{31}P_i$ was added to the system before incubation. This implies there is a direct transfer of the phosphoryl group of the substrate to ADP. However the results presented in the communication are not given in any detail and a full paper has never appeared. Durohydroquinone monophosphate, 2,3-dimethyl-4-hydroxy-naphthyl-1-phosphate and menadiol 1-phosphate have been shown to be oxidised by beef heart mitochondria and this oxidation is stimulated by cytochrome c (118).

THE ERICKSON SCHEME

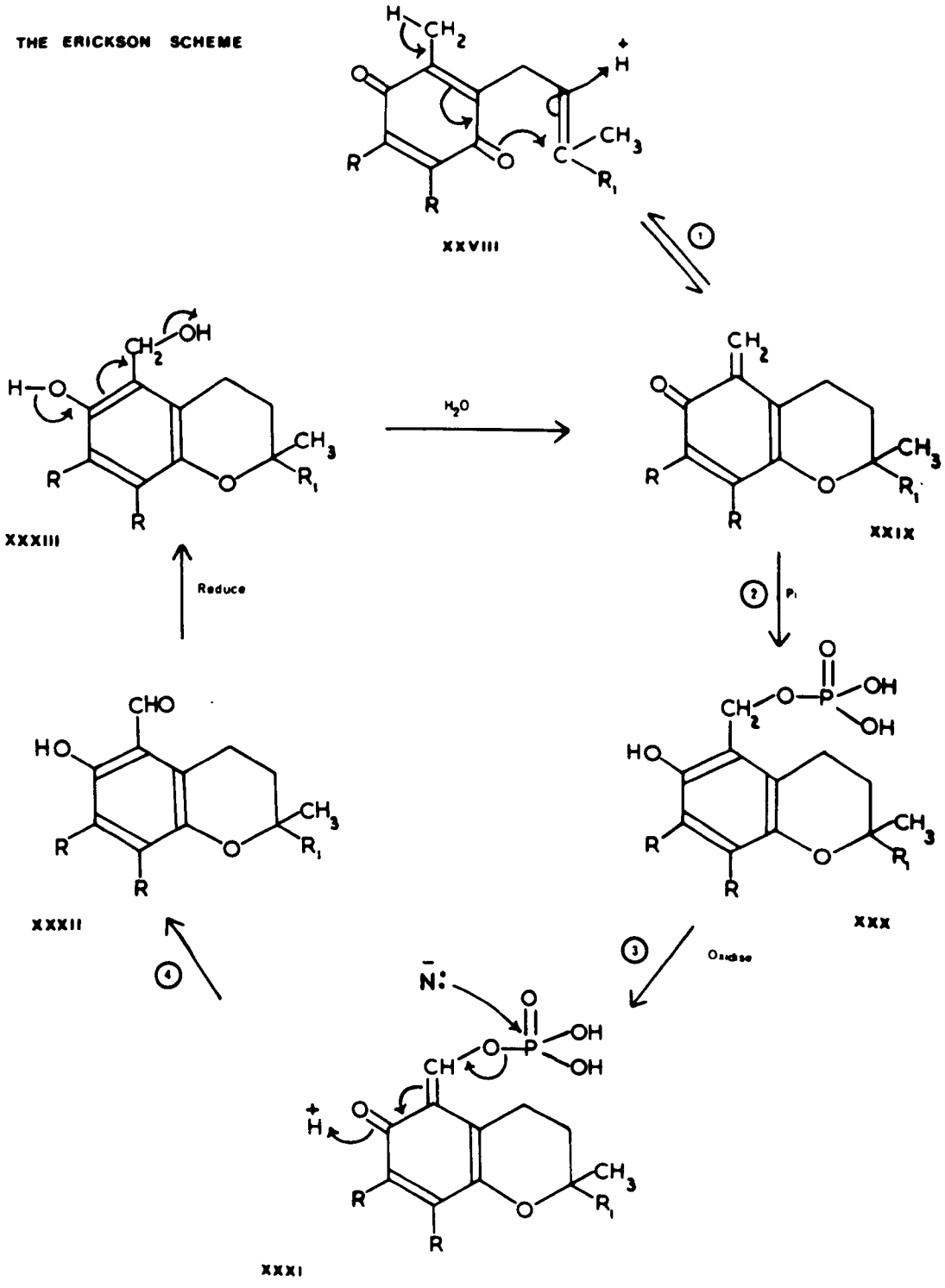


Figure 14

Present evidence shows that the hydroquinone phosphates and chromanyl phosphates may be oxidised by systems capable of oxidative phosphorylation but as yet there is no unequivocal evidence to show that there is a direct transfer of the phosphoryl group of a hydroquinone phosphate or a chromanyl phosphate to ADP.

A scheme for oxidative phosphorylation, which embraces all the advantages of the Vilkas-Lederer scheme but avoids its two thermodynamically unfavourable reactions, has been proposed by Erickson, Wagner, and Folkers (119) hereafter referred to as "The Erickson Scheme".

THE ERICKSON SCHEME (figure 14)

Isomerisation of the quinone, XXVIII, leads to the quinone methide, XXIX, which reacts with inorganic phosphate in a 1,4-addition reaction to form the benzyl phosphate, XXX. Oxidation of the benzyl phosphate, XXX, produces the quinone methide phosphate, XXXI, which eliminates metaphosphate or is attacked by a nucleophile to form the aldehyde, XXXII. This is the actual phosphorylation reaction. Reduction of the aldehyde gives the alcohol, XXXIII, which can then eliminate water to regenerate the quinone methide, XXIX.

In this scheme only a small portion of the quinone pool need be participating in oxidative phosphorylation. This could explain the negative results of the hydrogen isotope exchange experiments done in vivo. The reduced phosphorylated derivative of vitamin K_1 obtained by Brodie (112) may be a benzyl phosphate of the type, XXX (figure 14), but there is no experimental evidence to confirm this.

An in vitro investigation of the Erickson scheme for oxidative phosphorylation is the subject of the present work. In particular the first four reaction steps of the scheme were investigated.

OTHER THEORIES

Although present evidence strongly suggests that quinones are involved in electron transport, there is no unequivocal evidence to show that they are involved in the coupling reaction. Other mechanisms have been suggested by various different experiments.

Phosphorylated proteins may be the primary high-energy intermediate involved in oxidative phosphorylation. A phosphorylated form of a phosphoryl transferase has been isolated from beef heart mitochondria and increases the phosphorylative capacity of poorly phosphorylating submitochondrial particles (120).

ATP is produced in some 25% yield when a 5-methyl-mono-thiohydroquinone or N-acetyl-homocysteinthiolactone is oxidised by bromine in dry pyridine in the presence of ADP and P_i (121, 122). Blank reactions in which the sulphur compound is omitted yield ATP in only 0.5% yield, presumably *via* a pyridinium bromide intermediate (123). These reactions can serve as a model system for oxidative phosphorylation.

Mitchell (124) has developed a "Chemiosmotic" theory in which respiration and ATP synthesis are coupled *via* respiration-dependent proton transport through a membrane. This proton transport develops a pH gradient across the membrane as well as an electrochemical potential difference whose free energy corresponds to the high-energy intermediate of the chemical hypotheses. With a backward flow of protons across the membrane, the free energy of the electrochemical potential drives the elimination of water between ADP and P_i *via* a vectorial ATPase system which is bound to the membrane. The mechanism for this ATP synthesis is vague and is a weak point of the theory. Uncouplers are considered to act by increasing the permeability of the membrane to protons and there is evidence to this effect (125, 126, 127). Furthermore oxidation of substrates and

hydrolysis of ATP both lead to the expulsion of protons from mitochondria (128, 129) but more recent investigations suggest this may be a secondary reaction of a respiratory-coupled transport of calcium or potassium ions (130, 131).

RESULTS AND DISCUSSION

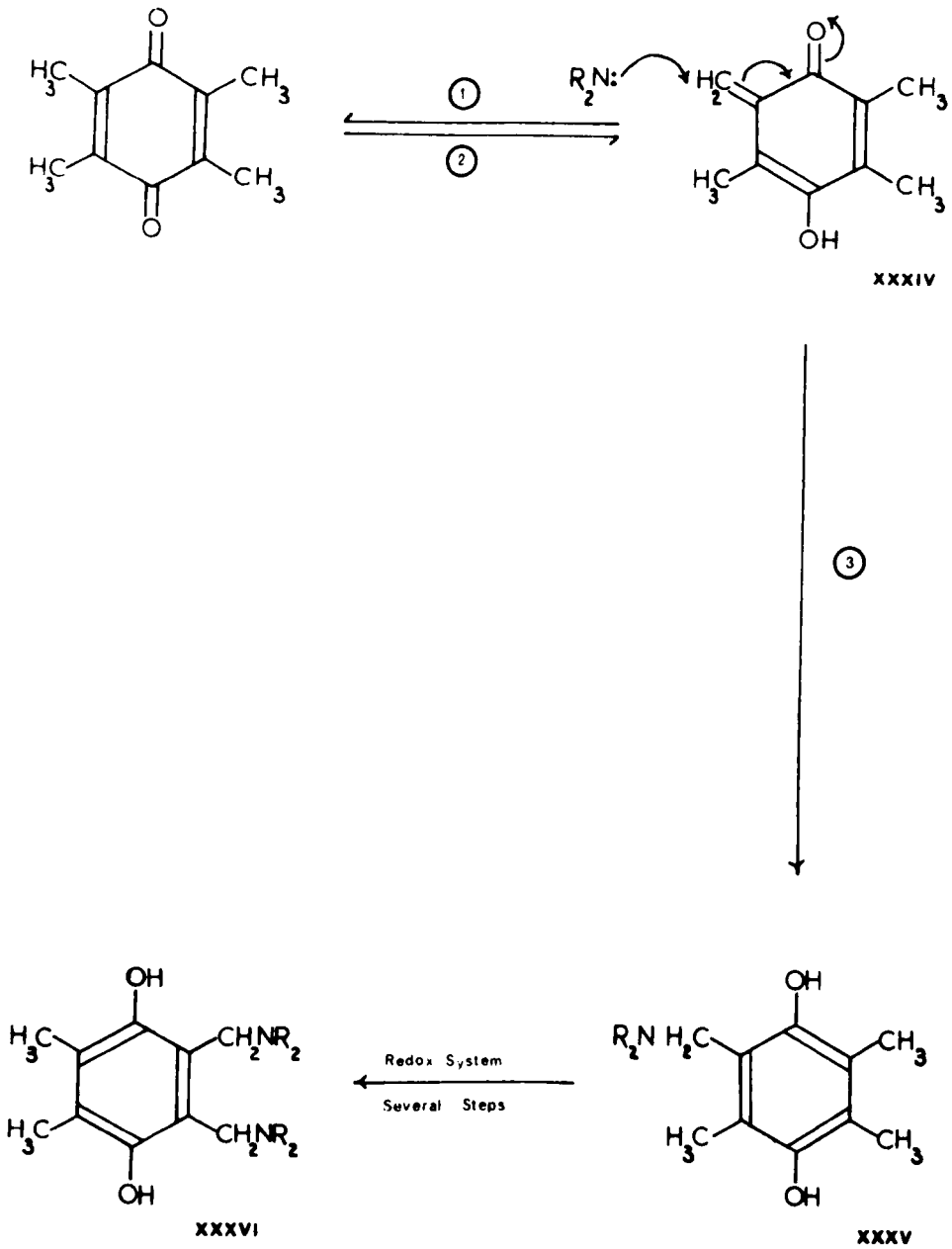

H3C
CH3
H3C
CH3
O
O
1
2
R2N:
H2C
O
CH3
H3C
CH3
OH
XXXIV
3
OH
H3C
CH2NR2
H3C
CH2NR2
OH
XXXVI
Redox System
Several Steps
R2N H2C
OH
CH3
H3C
CH3
OH
XXXV

figure 18

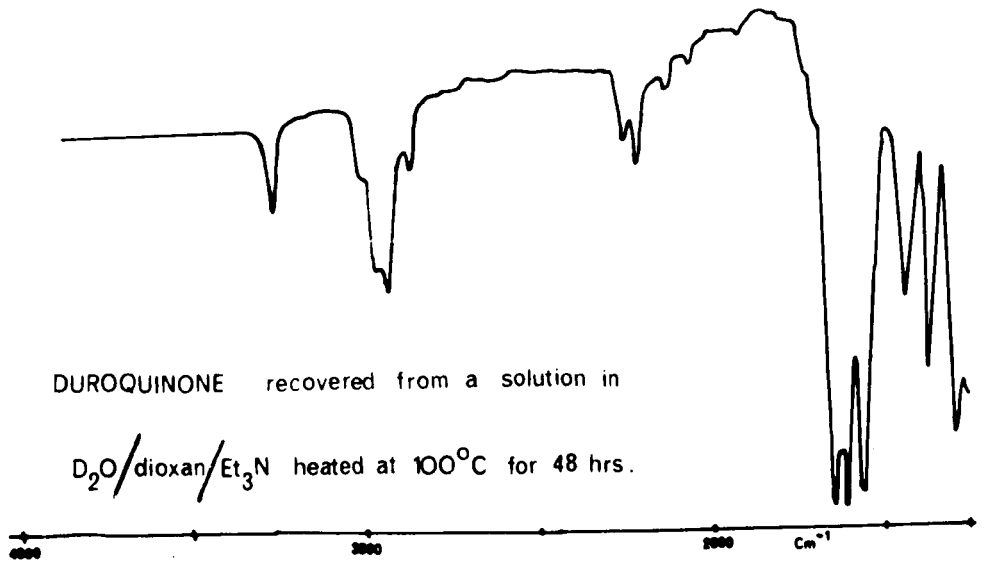
HYDROGEN ISOTOPE EXCHANGE IN METHYL QUINONES

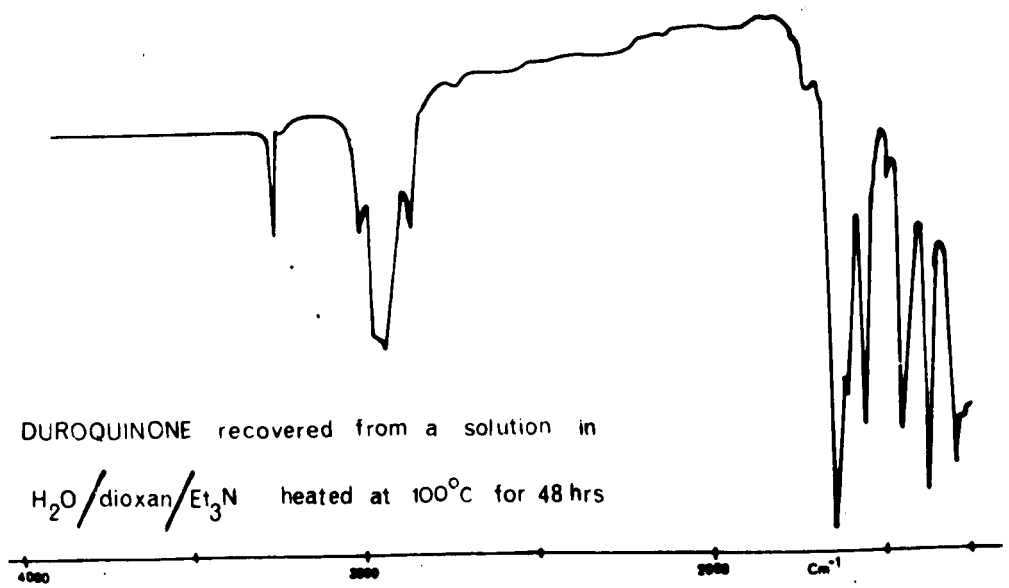
Hydrogen isotope exchange in methyl quinones was investigated in an attempt to provide evidence for quinone methides as intermediates - in particular for reaction 1 of the Erickson scheme for oxidative phosphorylation (figure 14).

The quinone methide tautomeric with a methyl quinone is analogous to the enolic form of an α,β-unsaturated ketone. Hydrogen isotope exchange in systems of the latter type is well known under both acidic and basic conditions (132 - 135). However, hydrogen isotope exchange has not been observed in systems of the former type despite considerable effort (see introduction p. 28). The reason for this is the unique nature of the quinone methide anion which can undergo both nucleophilic and electrophilic attack at the methylene carbon atom, as expected from its two canonical forms, XXXVI and XXXVIII (figure 17). The high nucleophilic reactivity of the actual resonance hybrid has precluded the observation of any hydrogen isotope exchange.

Duroquinone, which contains but one type of carbon-hydrogen bond, was selected for an initial investigation of hydrogen isotope exchange in methyl quinones. The side chain amination of duroquinone had been reported to proceed via a quinone methide intermediate (figure 15. Ref. 90). In the presence of a secondary

amine, duroquinone forms the quinone methide, XXXIV, which then reacts with the secondary amine in a 1,4-addition reaction to give the monosubstituted durohydroquinone, XXXV. This is then oxidised to the quinone and undergoes a further similar side chain amination to give the disubstituted durohydroquinone, XXXVI, as the final product. In the presence of an N-deuterated secondary amine, the proposed tautomeric equilibrium between the quinone and quinone methide should be demonstrable by observation of deuterium exchange in the duroquinone. However, no isotope exchange would be observed if reaction three were very fast compared to reaction two (figure 15). The overall rates of the reaction using morpholine, piperidine and pyrrolidine were found to be in the approximate ratio 1:60:600. Assuming the rapid pre-equilibrium shown in figure 15, this reflects a difference in the rate of reaction three. For the deuteration experiments N-deuteromorpholine was chosen as the base in order to favour reaction two over reaction three and thus the observation of hydrogen isotope exchange. However, when duroquinone was treated with N-deuteromorpholine for two days at room temperature, neither the recovered quinone nor the disubstituted product contained deuterium as indicated by their infra red spectra. This was in agreement with subsequent similar experiments by other





INFRA RED SPECTRA

figure 16

workers (89). It was concluded that either the reaction did not involve a quinone methide or that reaction one was the rate determining step of the reaction and reaction three was very fast, precluding the observation of reaction two (figure 15). Use of a base strong enough to remove a proton from the methyl group of the quinone but unable to react with the quinone methide produced would favour the back reaction and the observation of hydrogen isotope exchange; hence carbonate and triethylamine were the bases chosen. Deuterium oxide was used as the deuteron (D^+) source and dioxan as solvent.

A solution of duroquinone in dioxan/deuterium oxide containing potassium carbonate or triethylamine was heated under reflux for two days. The reaction mixture was then cooled and acidified to stop further reaction. Duroquinone could be recovered in about 1% yield by preparative thin layer chromatography. The infra red spectrum of the recovered duroquinone showed carbon-deuterium stretching vibrations (136) at 2250, 2210, 2130, 2060 cm^{-1}. (figure 16). Diduroquinone could be recovered from the same reaction mixture in more than 90% yield and its infra red spectrum showed carbon-deuterium stretching vibrations at 2240, 2200, 2110, 2060 cm^{-1}. However the intensities of the peaks were too weak for

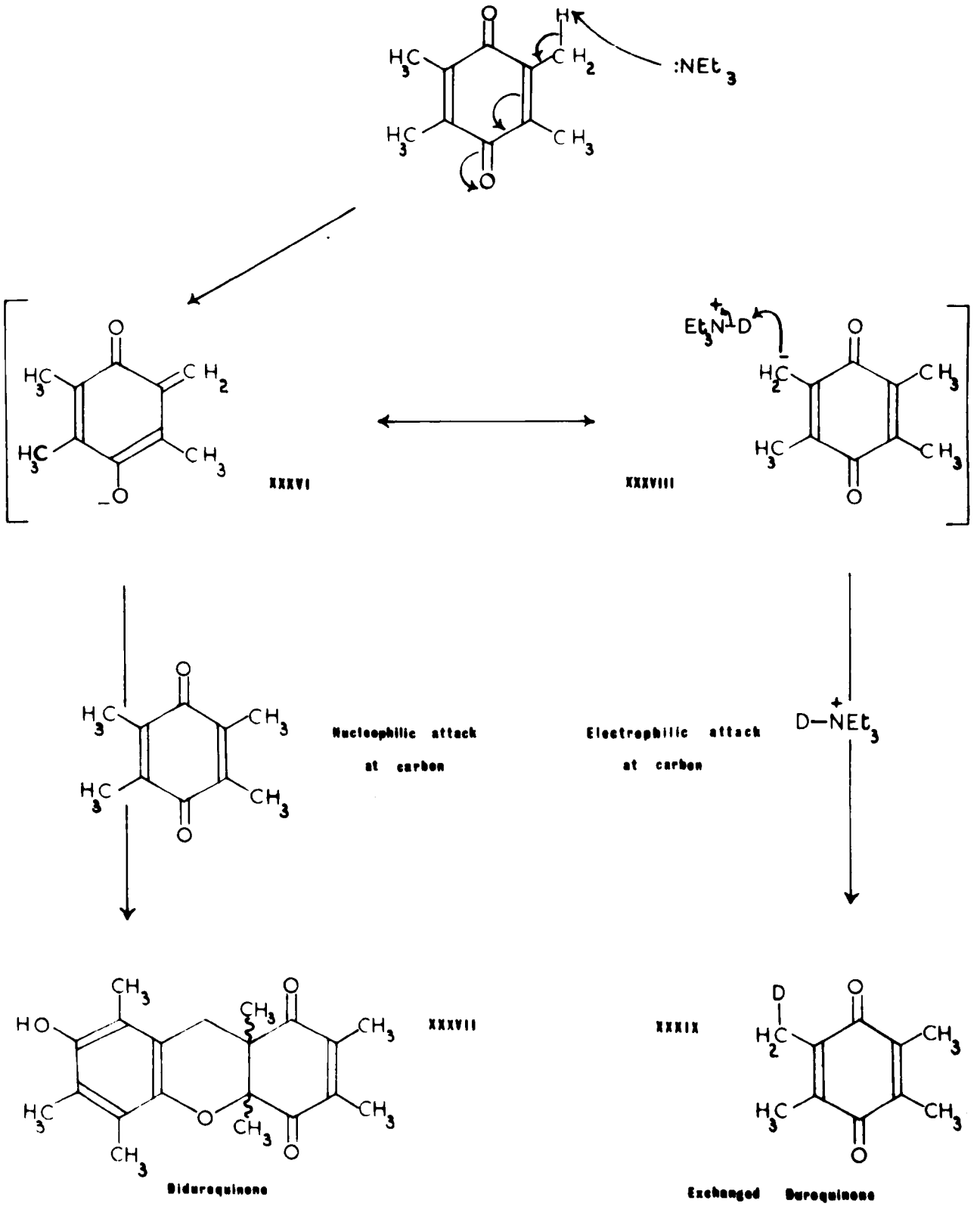
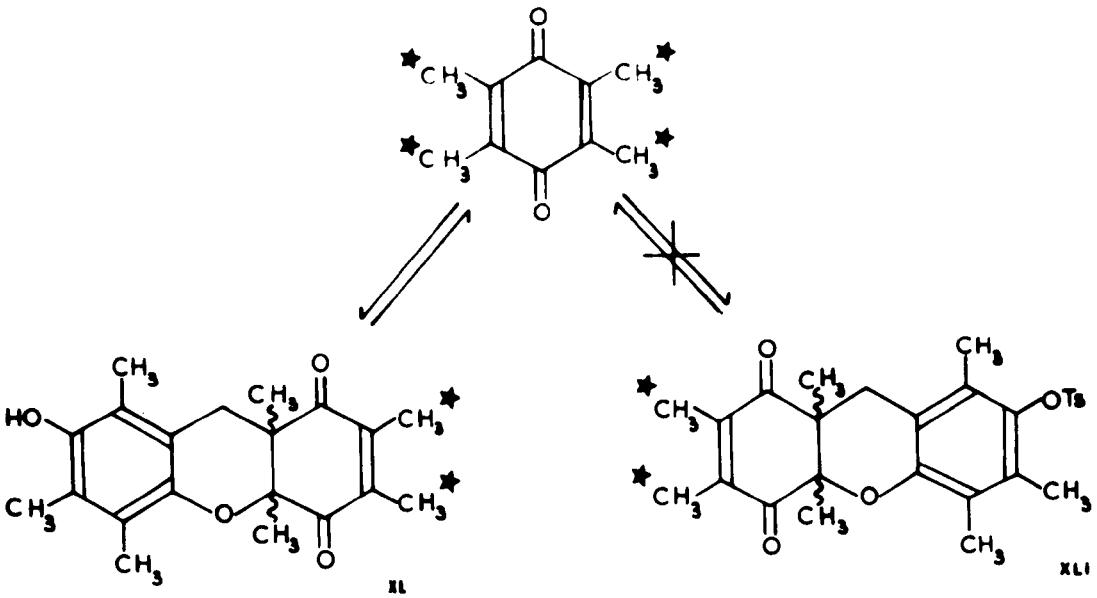

:NEt3
XXXVI
XXXVIII
Nucleophilic attack at carbon
Electrophilic attack at carbon
D–NEt3
XXXVII
XXXIX
Diduroquinone
Exchanged Duroquinone

figure 17

quantitative measurement of the deuterium incorporation. The conversion of most of the duroquinone to diduroquinone is easily rationalised when one considers the quinone methide anion formed by loss of a proton from the duroquinone. It is a resonance hybrid of the two canonical forms, XXXVI and XXXVIII (figure 17). Nucleophilic attack on the quinone methide anion at the methylene carbon atom by another molecule of duroquinone gives diduroquinone, XXXVII. Electrophilic attack on the quinone methide anion at the methylene carbon atom by a deuteron (D^+) forms deuterated duroquinone, XXXIX. Oxygen is much more electronegative than carbon and most of the charge in the resonance hybrid is localised on the oxygen atom. This results in a high nucleophilic reactivity for the quinone methide anion, most of which is therefore converted to diduroquinone.

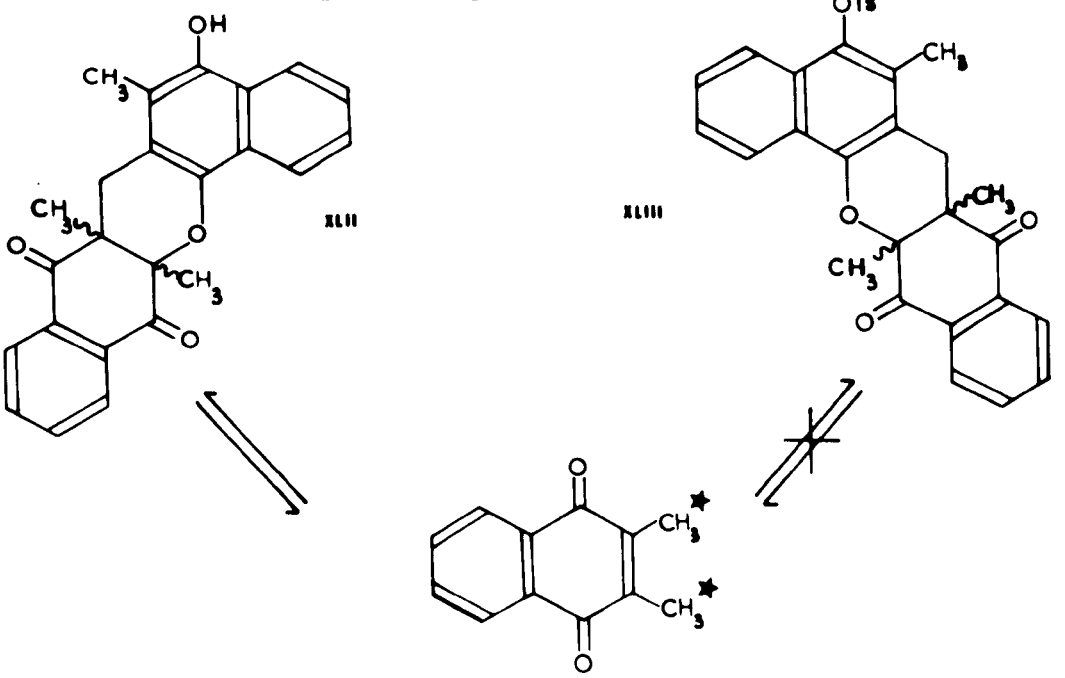
In order to measure the isotope exchange of the above reaction quantitatively, a technique for observing tritium exchange was developed. Progress curves for tritium uptake were determined. A solution of duroquinone in dioxan/tritiated water/triethylamine was heated at a constant temperature in sealed ampoules. Samples were removed at suitable intervals and each was cooled and acidified to stop further reaction. The duroquinone was separated



XL XLI

figure 18(a)

* = exchangeable hydrogen



XLII XLIII

figure 18(b)

from the reaction mixture and purified by preparative thin layer chromatography. Extraction of the duroquinone from the final thin layer chromatogram with spectroscopically pure dioxan gave a solution whose concentration was measured by ultra violet spectroscopy. The amount of tritium in this solution was determined by mixing a known volume of the solution with a scintillator solution and counting the resultant scintillations with a liquid scintillation spectrometer. Duroquinone has strong quenching properties in common with many other coloured compounds (137). It was therefore necessary to count very small quantities of duroquinone and to correct the observed counts for this quenching effect (quenching curves - figure 34). If the recovered quinone has x counts/second/mole of exchangeable hydrogen and the water of the stock solution has y counts/second/mole of hydrogen then the scrambling is defined as (x/y x 100)%.

When diduroquinone was heated under the conditions of the exchange experiments, a little duroquinone was formed. Hence the two compounds are in dynamic equilibrium under the conditions of the exchange reaction. It was therefore necessary to prove that a genuine exchange for duroquinone was being observed and not an exchange in diduroquinone followed by a reformation of monomeric

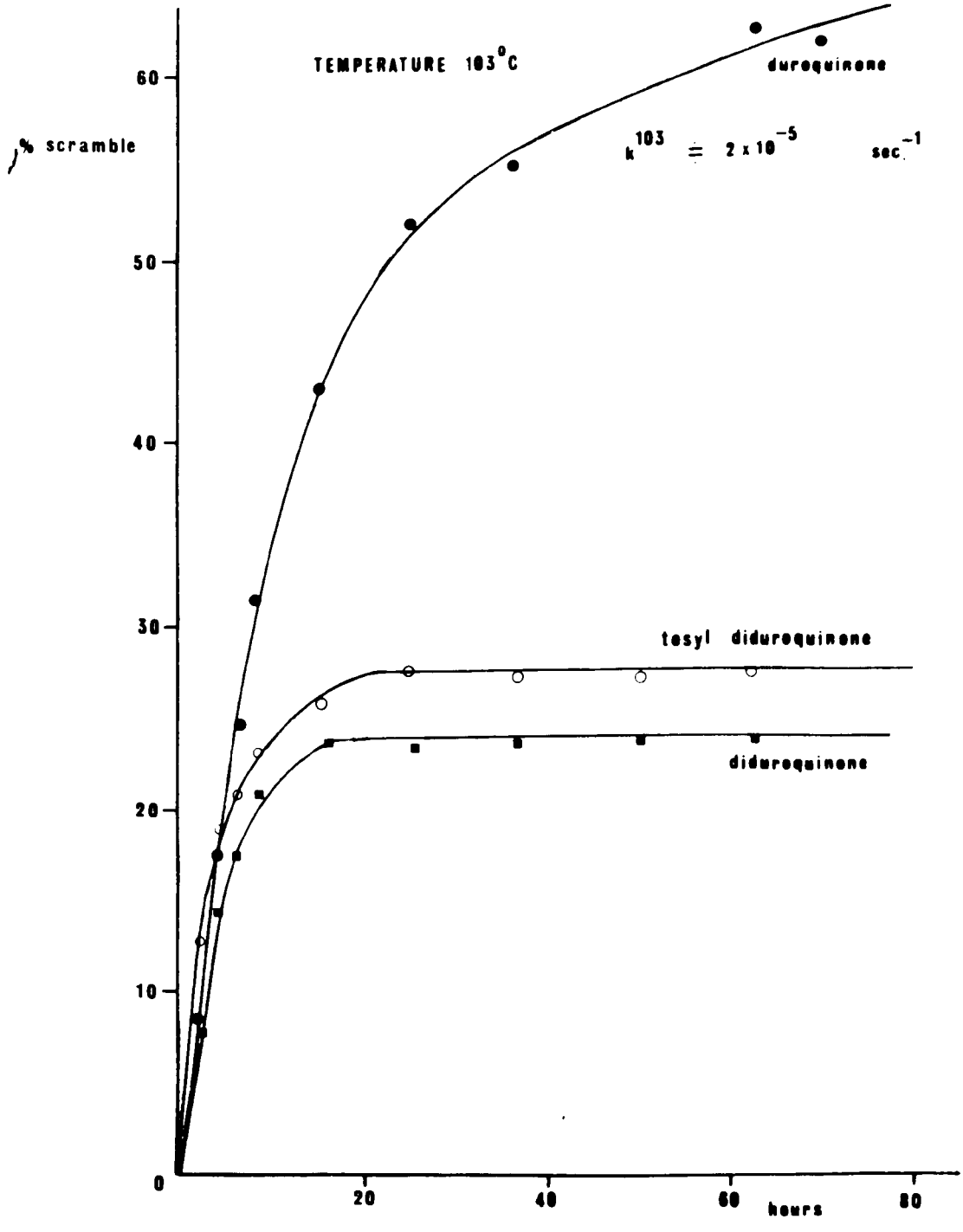

TEMPERATURE 103°C
durequinone
$k^{103} = 2 \times 10^{-5}$ sec^{-1}
% scramble
60
50
40
30
20
10
0
tesyl diduroquinone
diduroquinone
20
40
60
hours
80

figure 19

duroquinone (figure 18a). Diduroquinone, XL, contains six exchangeable hydrogens - complete exchange corresponds to 25% of the total hydrogens. Esterification or alkylation of the phenolic hydroxyl group in diduroquinone blocks its dynamic equilibrium with duroquinone (figure 18a). This enables the exchange reaction of a diduroquinone derivative to be studied in isolation - the tosyl ester of diduroquinone, XLI, being suitable as it is stable under the exchange conditions.

Solutions of duroquinone, diduroquinine, and the tosyl ester of diduroquinone in dioxan/tritiated water/triethylamine were heated at 103^{o}C in sealed ampoules. Tritium incorporation into each of the samples, taken at varying time intervals, was determined by the usual sequence of chromatographic and spectroscopic techniques. The progress curves are shown in figure 19. Duroquinone continues to undergo exchange after diduroquinone and the tosyl ester of diduroquinone have exchanged completely - demonstrating a genuine exchange in duroquinone. The pseudo first order rate constant for the three exchange reactions at 103^{o}C is of the order of 2×10^{-5} sec^{-1}.

The hydrogen isotope exchange of the 2,3-dimethyl-1,4-naphthoquinone system was studied in some detail. The dimer of

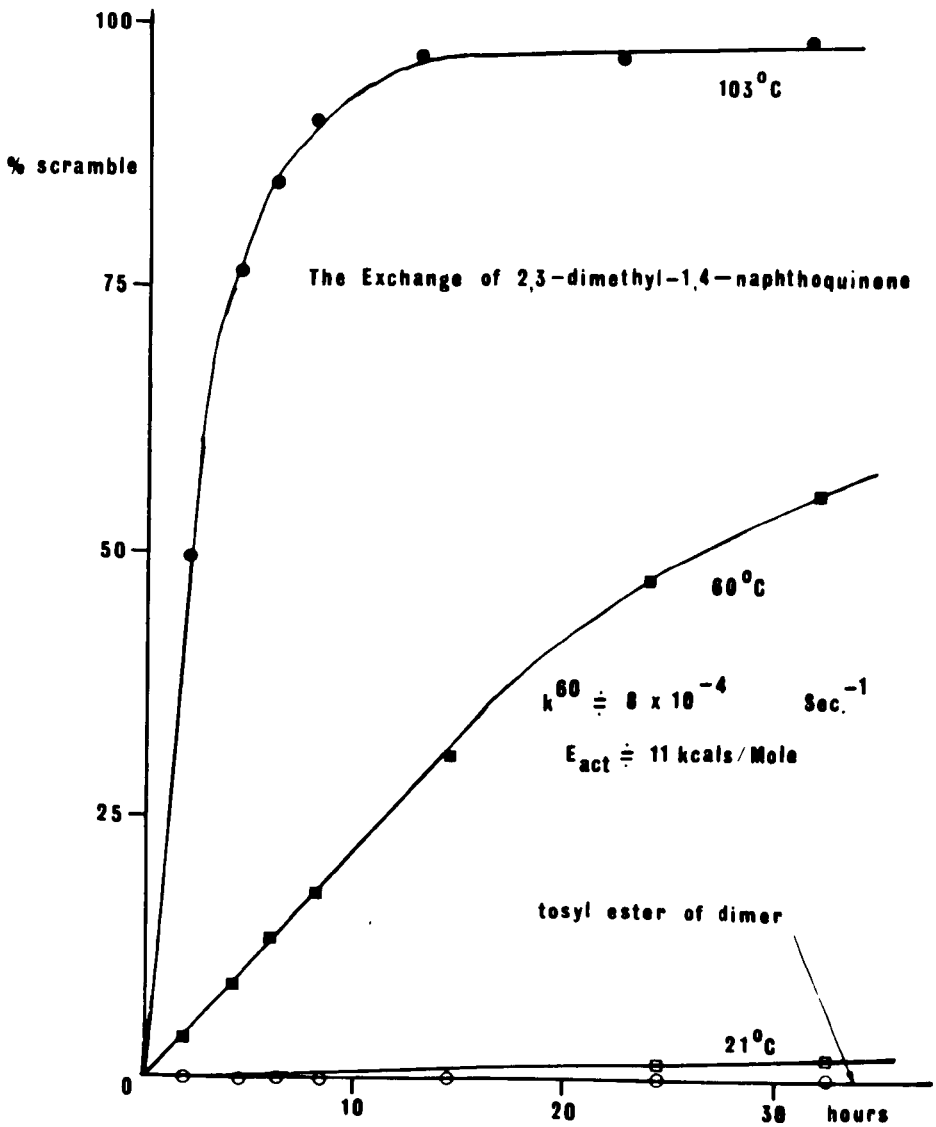

100
% scramble
75
50
25
0
10
20
30
hours
103°C
The Exchange of 2,3-dimethyl-1,4-naphthoquinone
60°C
$k^{60} \doteq 8 \times 10^{-4}$ Sec.$^{-1}$
$E_{act} \doteq 11$ kcals/Mole
tosyl ester of dimer
21°C

figure 20

2,3-dimethyl-1,4-naphthoquinone, XLII (figure 18b), contains no exchangeable hydrogens and exchange can therefore occur in the monomeric quinone only. Under the exchange conditions most of the dimer is converted to the monomeric quinone and as a result 2,3-dimethyl-1,4-naphthoquinone could be recovered from the exchange reaction mixtures in about 30% yield. As for diduroquinone, alkylation or esterification of the phenolic hydroxyl group of the dimer of 2,3-dimethyl-1,4-naphthoquinone blocks its dynamic equilibrium with the monomeric quinone, and the tosyl ester of the dimer of 2,3-dimethyl-1,4-naphthoquinone, XLIII (figure 18b), being stable under the exchange conditions, provides a suitable derivative with which to study the isotope exchange reaction in isolation. The exchange of 2,3-dimethyl-1,4-naphthoquinone and the tosyl ester of its dimer was investigated at various temperatures using a method analogous to that described for duroquinone and its derivatives, and the results are indicated in figure 20. At 103°C scrambling of the tritium between the water and the quinone is almost complete after 12 hours. The pseudo first order rate constant for the reaction at 60°C is of the order of 8×10^{-4} sec.$^{-1}$. The Arrhenius activation energy (138) for the reaction is of the order of 11 kcals.

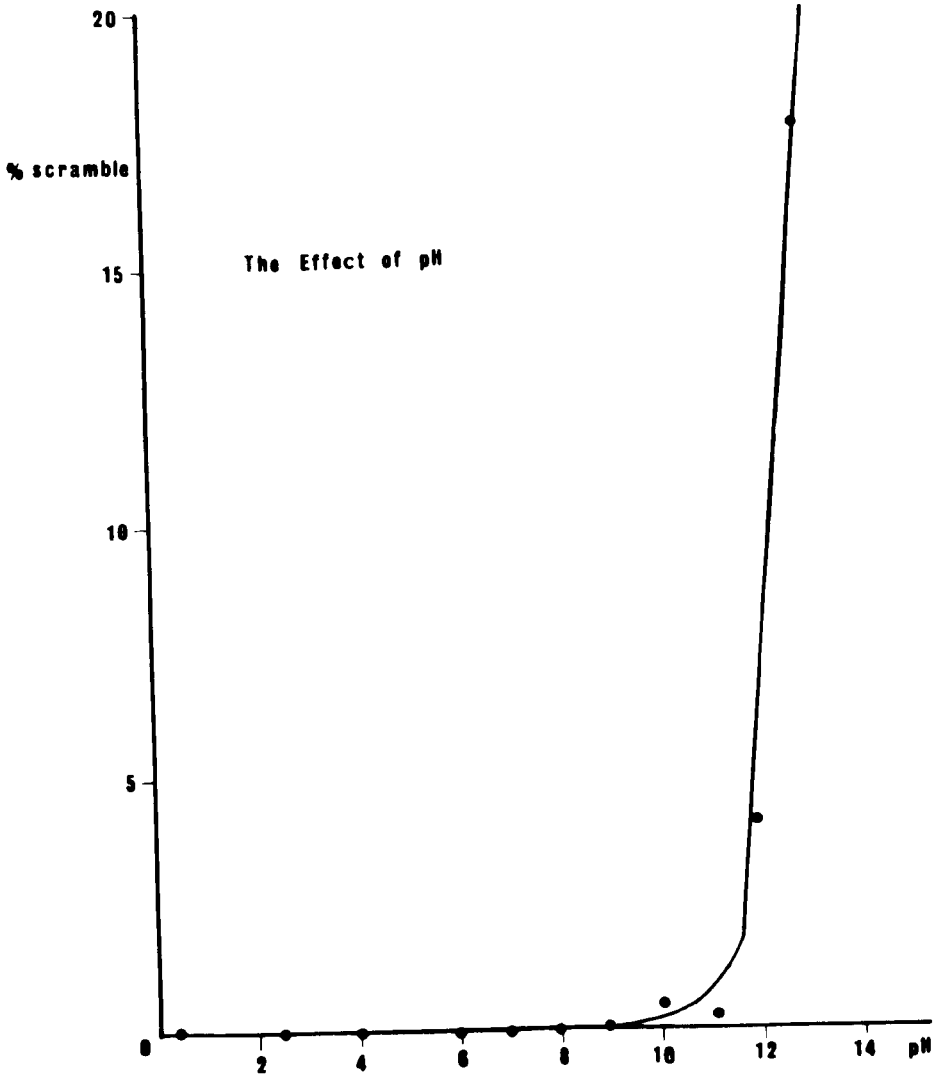


figure 21

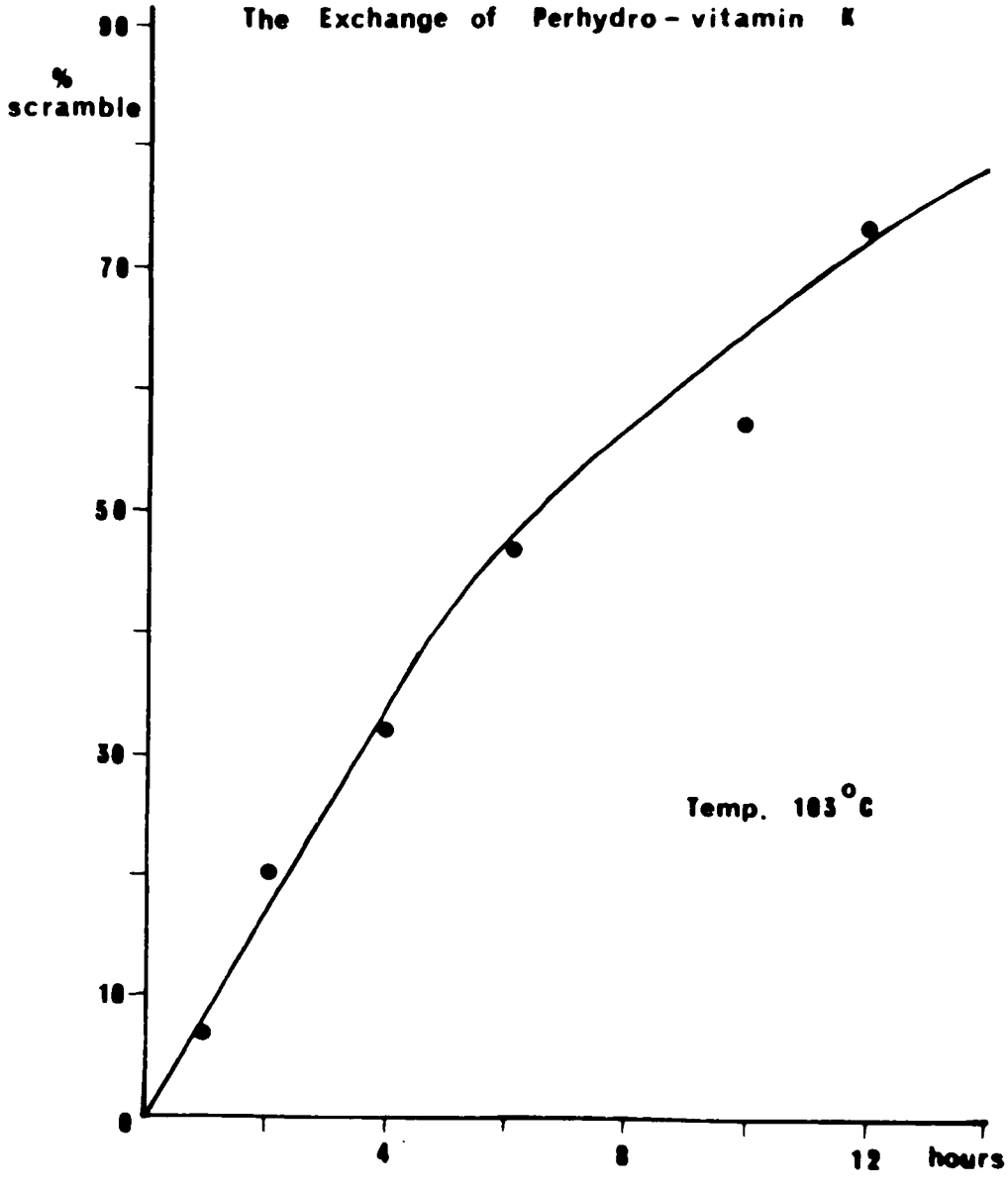

The Exchange of Perhydro - vitamin K
% scramble
80
70
50
30
10
0
4
8
12
hours
Temp. 183°C

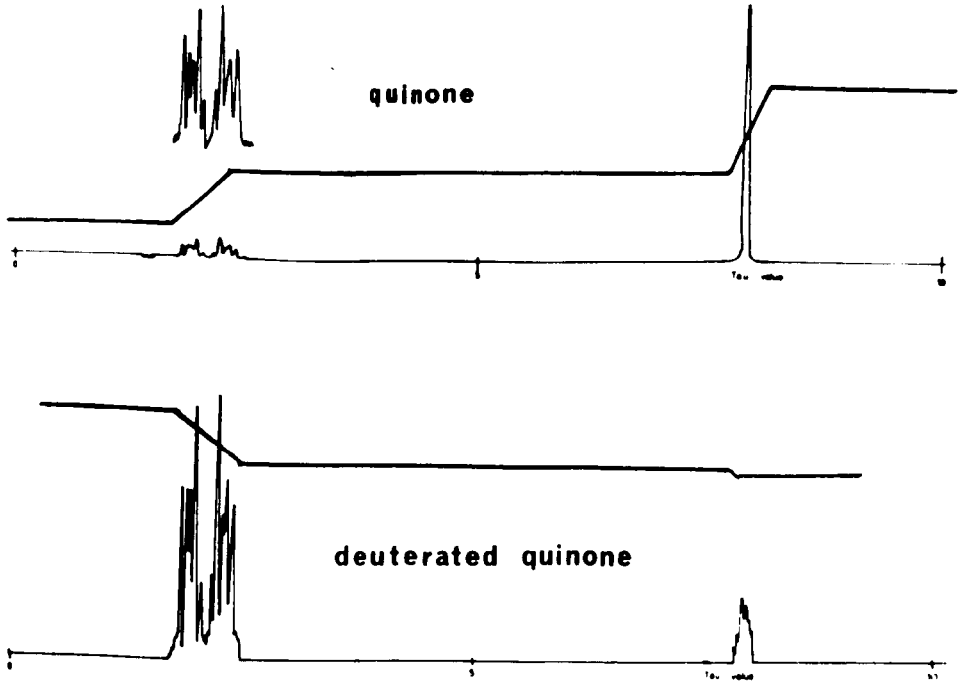
figure 22

Mole^{-1}. The tosyl ester of the dimer of 2,3-dimethyl-1,4-naphthoquinone did not exchange to an appreciable extent.

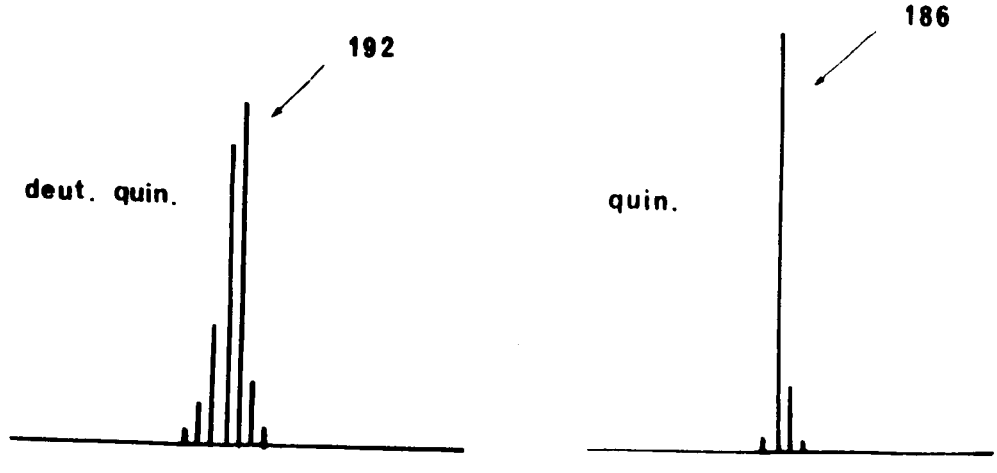
The above reaction was investigated over a range of apparent pH (139). The apparent pH of the buffers used, which were based on those of Britton and Robinson (140) but made up in dioxan/water, was measured using a glass electrode pH meter, the reading of the meter defining the apparent pH. The results of the experiment are indicated in figure 21. While there was an exponential increase in the observed isotope exchange with increasing pH above pH 9, virtually no exchange occurred below pH 9.

Vitamin K_1 was found to be unstable under the exchange conditions, but tritium uptake into perhydro-vitamin K_1 was readily observed. The experiment was carried out using the normal procedure and the results are indicated in figure 22. 75% scrambling of the tritium between the water and the perhydro-vitamin K_1 occurred in 13 hours at 103^{o}C.

The deuterium content of 2,3-dimethyl-1,4-naphthoquinone, recovered from similar exchange experiments in dioxan/deuterium oxide, could be measured quantitatively using mass spectrometry. The mass spectrum of 2,3-dimethyl-1,4-naphthoquinone shows the parent molecular ion at m/e 186 (Ref. 141). Depending on the degree of deuteration, the group of parent molecular ions in



a) NMR SPECTRA



b) MASS SPECTRA

figure 23

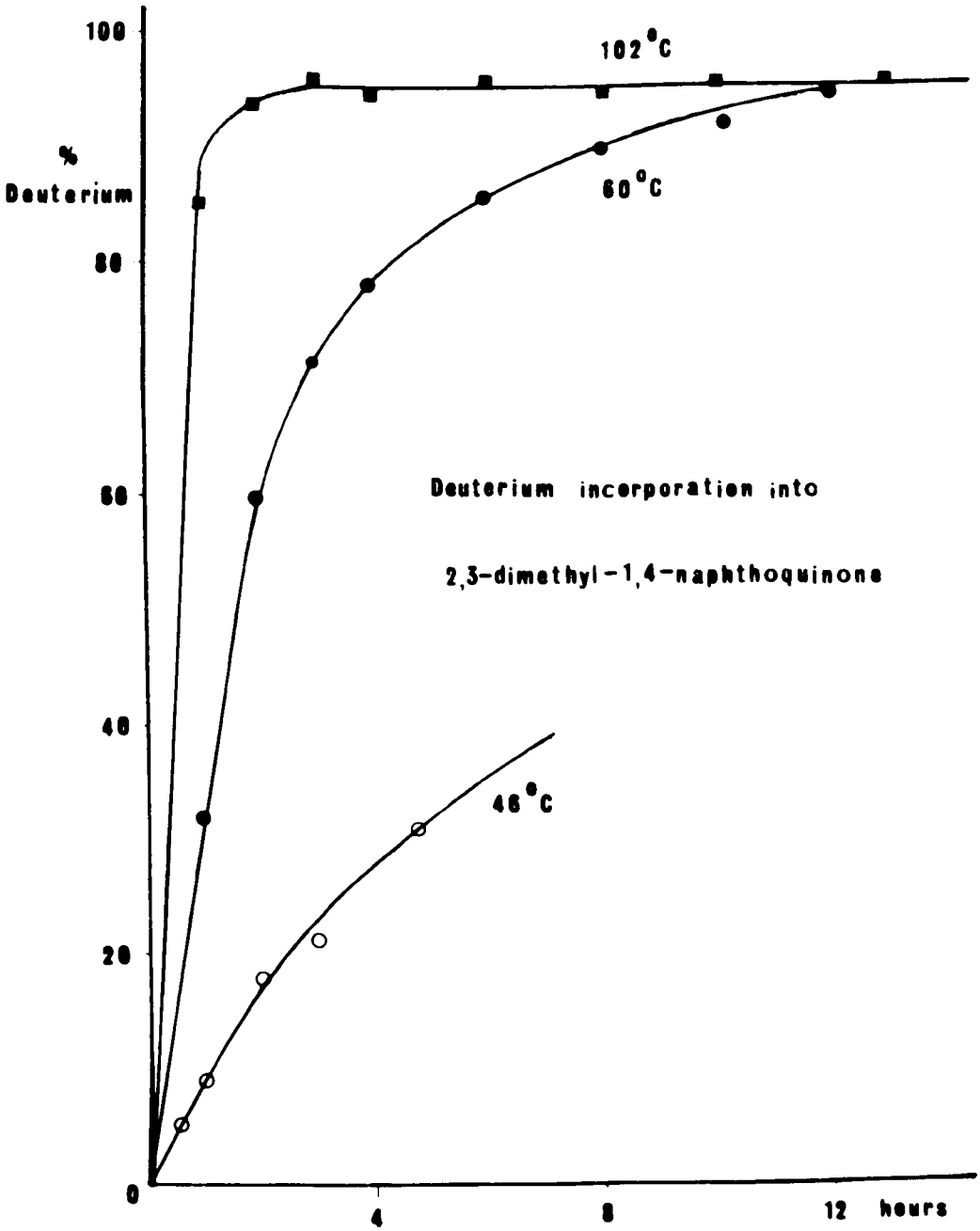

100
%
Deuterium
80
60
40
20
0
102°C
60°C
46°C
Deuterium incorporation into
2,3-dimethyl-1,4-naphthoquinone
4
8
12 hours

figure 24

the mass spectrum of a deuterated quinone sample may show peaks over the range m/e 186 - 192, corresponding to the undeuterated through to the fully deuterated quinone (figure 23b). The actual deuterium incorporation could be calculated from the heights of the peaks in this group of parent molecular ions and agreed to within 5% of that calculated from the NMR spectrum (figure 23a). The methyl signal in the NMR spectrum of the undeuterated quinone sample appears as a sharp singlet, whereas in a highly deuterated quinone sample (figure 23a), the proton signal due to the CHD_2 group appears as a poorly resolved quintet because the proton is coupled to two equivalent deuterium atoms which have nuclear spin 1 and a coupling constant with hydrogen of about 2 c/s (Ref. 142). For the sample whose spectrum is shown in Figure 23(a), comparison of the area under the methyl and aromatic peaks shows the deuterium incorporation to be 95%. The results of a series of exchange reactions at different temperatures are shown in figure 24. At $103^{o}C$ about 95% deuterium incorporation occurs within two hours. The pseudo first order rate constant for the reaction at $60^{o}C$ is of the order of 6×10^{-5} sec.$^{-1}$ and the Arrhenius activation energy (138) is of the order of 11 kcals. mole^{-1}.

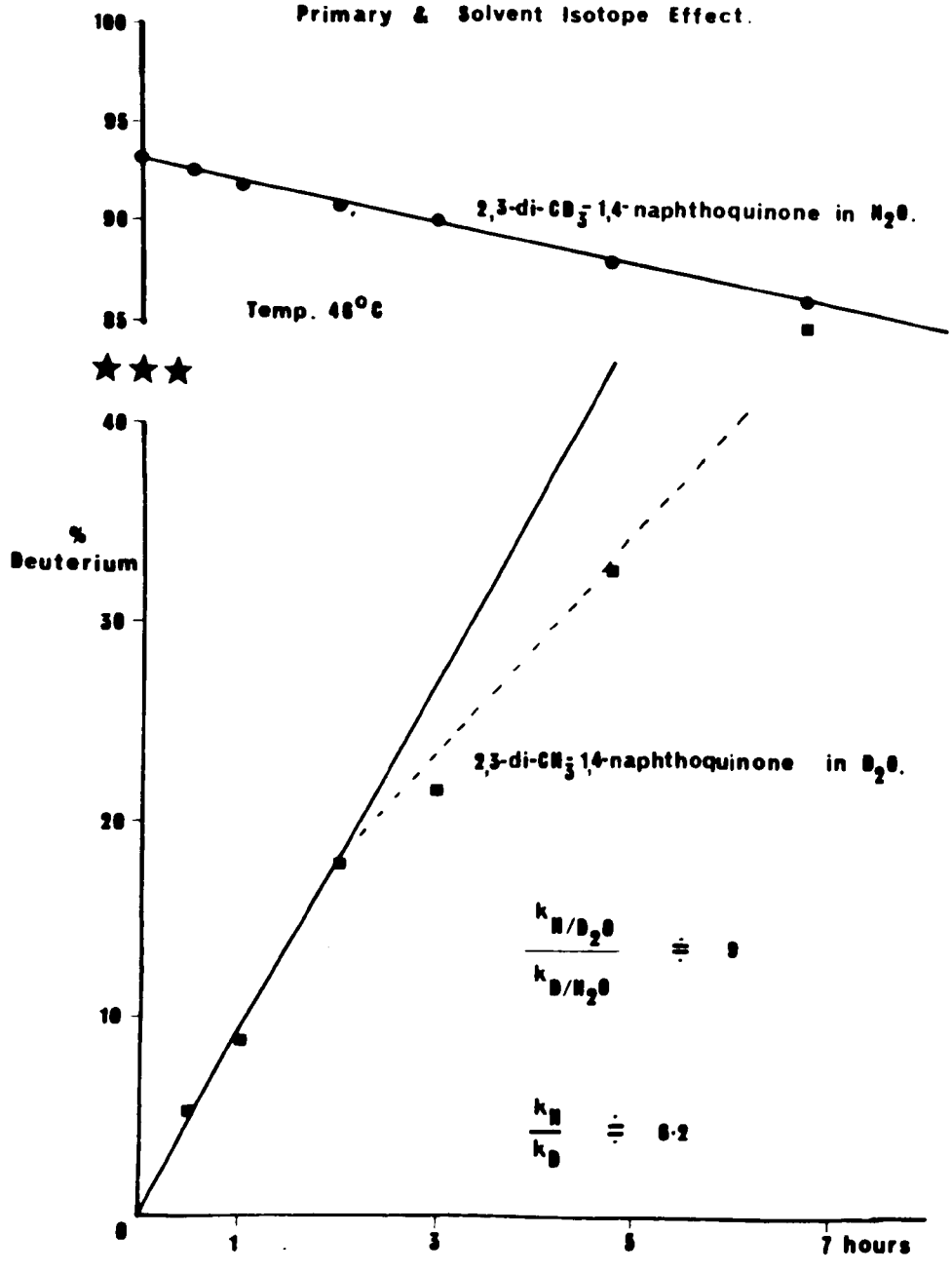

Primary & Solvent Isotope Effect.
100
95
90
85
2,3-di-CH3 1,4-naphthoquinone in H2O.
Temp. 48°C
40
% Deuterium
30
20
10
0
2,3-di-CH3 1,4-naphthoquinone in D2O.
k H/D2O / k D/H2O ≐ 9
k H / k D ≐ 8·2
1
3
5
7 hours

figure 25

In order to acertain if the removal of a proton from the methyl group of the quinone was the rate determining step for the exchange reaction, the isotope effect for the reaction was investigated (for reviews on the isotope effect see Ref. 143, 144). A solution of 2,3-dimethyl-1,4-naphthoquinone in dioxan/deuterium oxide/ triethylamine, and a solution of 2,3-di-perdeuteromethyl-1, 4-naphthoquinone (95% deuterated) in dioxan/protium oxide/ triethylamine, each in sealed ampoules, were heated at 46°C and the quinone extracted at suitable time intervals by the normal procedure. The deuterium content of the recovered samples of quinone was determined by mass spectrometry and the results are indicated in figure 25. The undeuterated quinone exchanged about 9 times faster than the deuterated quinone. The implications of this observation will now be discussed.

Because of the difference in zero-point energy a carbon-protium bond is more easily broken than a carbon-deuterium bond. When the formation of an activated transition state complex involves partial or total rupture of a carbon-hydrogen bond, the complex will be formed more easily from a carbon-protium than a carbon-deuterium bond, and the reaction rate will be faster for the protium compound than the deuterium compound. The observation

of such a difference in rates is evidence for the breaking or partial breaking of a carbon-hydrogen bond in the rate determining step of the reaction. This is called the "primary isotope effect" (144). The experiment in the present case is more complicated because there is also a solvent isotope effect, for protium oxide is used in one experiment and deuterium oxide in the other. Most base catalysed reactions are faster in deuterium oxide than in protium oxide (144). The mechanism of such a reaction is :

$$QH + B \rightleftharpoons BH^{+} + Q^{-} \xrightarrow{2} \text{products}$$

consider the competing reaction

$$D_2O + B \rightleftharpoons BD^{+} + OD^{-} \quad \text{(i)}$$

$$H_2O + B \rightleftharpoons BH^{+} + OH^{-} \quad \text{(ii)}$$

Since deuterium oxide has a smaller autoprotolysis constant than protium oxide by a factor of five (145), it is less acidic than protium oxide. Hence the base (triethylamine in the present case) can compete more effectively for the proton in the substrate (2, 3-dimethyl-1, 4-naphthoquinone in the present case) when the solvent is deuterium oxide than when it is protium oxide. Since the

concentration of the conjugate base of the substrate will be greater in deuterium oxide than in protium oxide, the overall reaction will be faster in deuterium oxide provided reaction two does not involve an isotope effect. In the present exchange reaction of 2,3-dimethyl-1,4-napthoquinone, $k_{H/D_2O} / k_{D/H_2O} = 9$ and this comprises both a primary and a solvent isotope effect. An approximate correction for the solvent isotope effect can be made by assuming it will be similar to that observed for the basic hydrolysis of diacetone alcohol which proceeds by a similar mechanism (146). For this reaction $k_{D_2O} / k_{H_2O} = 1.45$. Correcting the combined isotope effect for the exchange reaction by this factor gives a value of $k_H / k_D = 6.2$ for the primary isotope effect of the hydrogen isotope exchange reaction of 2,3-dimethyl-1,4-naphthoquinone at 46^oC. This corresponds to the maximum value at this temperature (144) and implies that the carbon-hydrogen bond must be almost totally broken in the transition state.

The results of this experiment show that the removal of a proton from the methyl group of the quinone is the rate determining step of reactions involving a quinone methide intermediate and

offers a possible explanation of the negative results of the in vivo experiments designed to investigate hydrogen isotope exchange in methyl quinones.

THE ATTEMPTED ADDITION OF PHOSPHATE TO A QUINONE METHIDE

The reaction of a phosphate with a quinone methide has been investigated in an attempt to provide evidence for reaction 2 of the Erickson scheme for oxidative phosphorylation (figure 14).

Quinone methides (general formula, XXIV, figure 11b) can be reguarded as quinones in which one doubly-bonded oxygen atom has been replaced by a methylene group (for reviews see Ref. 96, 97, 98). The carbonyl group has a large dipole moment and the positive character of the carbonyl carbon atom is partially transferred by electron delocalisation through the π-electron system of the quinone methide to the methylene carbon atom. This dipolar character, together with the energy obtained from the aromatisation of the ring, gives rise to greater reactivity towards nucleophiles than is observed with quinones, and in particular leads to nucleophilic addition or polymerisation at the methylene carbon atom. The reactions of methanol (99, 105) and acetic acid (102) with a quinone methide are examples of nucleophilic addition at the methylene carbon atom. Orthophosphoric acid or its anions, which are less acidic and hence more nucleophilic than the free

acid, might be expected to undergo similar nucleophilic addition with a quinone methide. As discussed earlier (page 33), the two claims in the literature (107, 108) to have observed such a reaction are not unequivocal.

In the present work duroquinone in dioxan was reacted with di-tetra-n-butyl-ammonium monohydrogen phosphate to see whether or not the phosphate dianion would be both basic enough to remove a proton from the methyl group of the quinone and then sufficiently nucleophilic, in its now monoanionic form, to react with the quinone methide anion produced to give a hydroxybenzyl phosphate derivative. Di-tetra-n-butyl-ammonium monohydrogen phosphate reacted rapidly with a solution of duroquinone in dioxan; however diduroquinone and polymeric material were the only products isolated. Paper chromatography showed that inorganic phosphate was the only phosphorus-containing compound present in the reaction mixture. It is presumed that dimerisation and polymerisation of the quinone methide anion intermediate is relatively fast. Change of temperature had little effect, similar results being obtained at room temperature and when the reaction mixture was heated under reflux ($100^{o}C$). Similar results were obtained using tri-tetra-n-butyl-ammonium phosphate but mono-tetra-n-

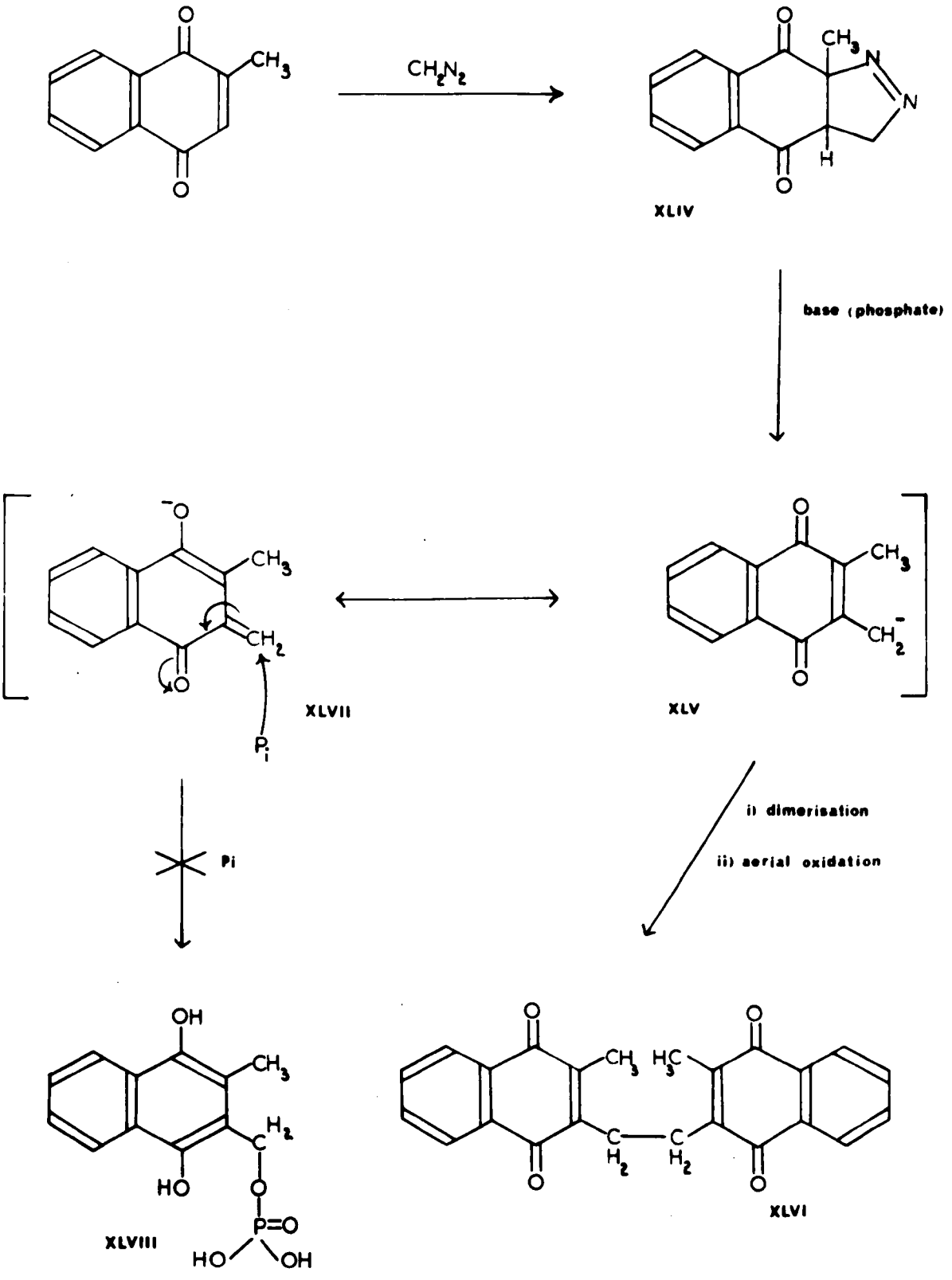
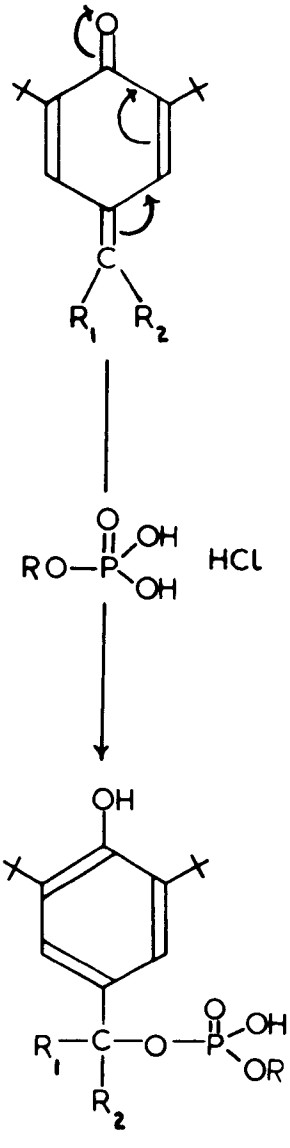

CH2N2
XLIV
base (phosphate)
XLVII
XLV
Pi
i) dimerisation
ii) aerial oxidation
XLVIII
XLVI

figure 26

butyl-ammonium dihydrogen phosphate was found to be not sufficiently basic to react with duroquinone, which could be recovered from the reaction mixture by TLC.

The anion of 2, 3-dimethyl-1, 4-naphthoquinone (XLVII, figure 26) has been produced under very mildly basic conditions from 3a, 4, 9, 9a-tetrahydro-9a-methyl-4, 9-dioxo-3H-benz(f)indazole (XLIV, figure 26) and has been trapped using ethyl coumarin-3-carboxylate to give a quinoylmethyldihydrocoumarin (100). We investigated the reaction of the dihydrogen phosphate monoanion with the indazole, XLIV, to see whether the addition of the phosphate to the quinone methide anion produced, could be observed. Dimethyl sulphoxide (review, see Ref. 147) was chosen as the non-aqueous solvent because it is a fairly good ionising solvent and specifically solvates cations rather than anions, hence enhancing the nucleophilicity of the phosphate monoanion.

Mono-tetra-n-butyl-ammonium dihydrogen phosphate reacted rapidly with a solution of the indazole, XLIV, in dimethyl sulphoxide and a brisk evolution of nitrogen was observed. However, the ethylene diquinone, XLVI, and other polymeric material were the only isolable products. Paper chromatography showed inorganic phosphate as the only phosphorus-containing compound present in the reaction mixture. It is presumed that the dimerisation and



XLIX

L

figure 27

polymerisation reactions are sufficiently fast to preclude any nucleophilic attack by phosphate on the quinone methide anion, XLVII, to give the hydroxybenzyl phosphate, XLVIII, if indeed any such reaction can occur. Similar results were obtained when di- or tri-tetra-n-butyl-ammonium phosphate was used as the base and DMF, dioxan, or acetonitrile as the solvent.

In an American Patent (107), Thompson claims to have observed the addition of both mono- and di-octyl orthophosphoric acid to 2,6-di-t-butyl-4-isopropylidene-2,5-cyclohexadien-1-one, XLIX ($R_1 = R_2 = CH_3$; figure 27) in benzene saturated with anhydrous hydrogen chloride; no details of the 3,5-di-t-butyl-4-hydroxy-α,α-dimethyl-benzyl phosphate product, L ($R_1 = R_2 = CH_3$; figure 27), were given. In the present work a very similar reaction using phenyl phosphoric acid in place of octyl phosphoric acid was investigated. Several products were produced. They could not be completely identified, although paper chromatography showed that monophenyl phosphoric acid was the only phosphorus-containing compound present in the reaction mixture. Similar results were obtained using 2,6-di-t-butyl-4-ethylidene-2,5-cyclohexadien-1-one (XLIX; $R_1 = H$, $R_2 = CH_3$; figure 27). When the reaction was carried out in the absence of hydrogen chloride similar results were observed.

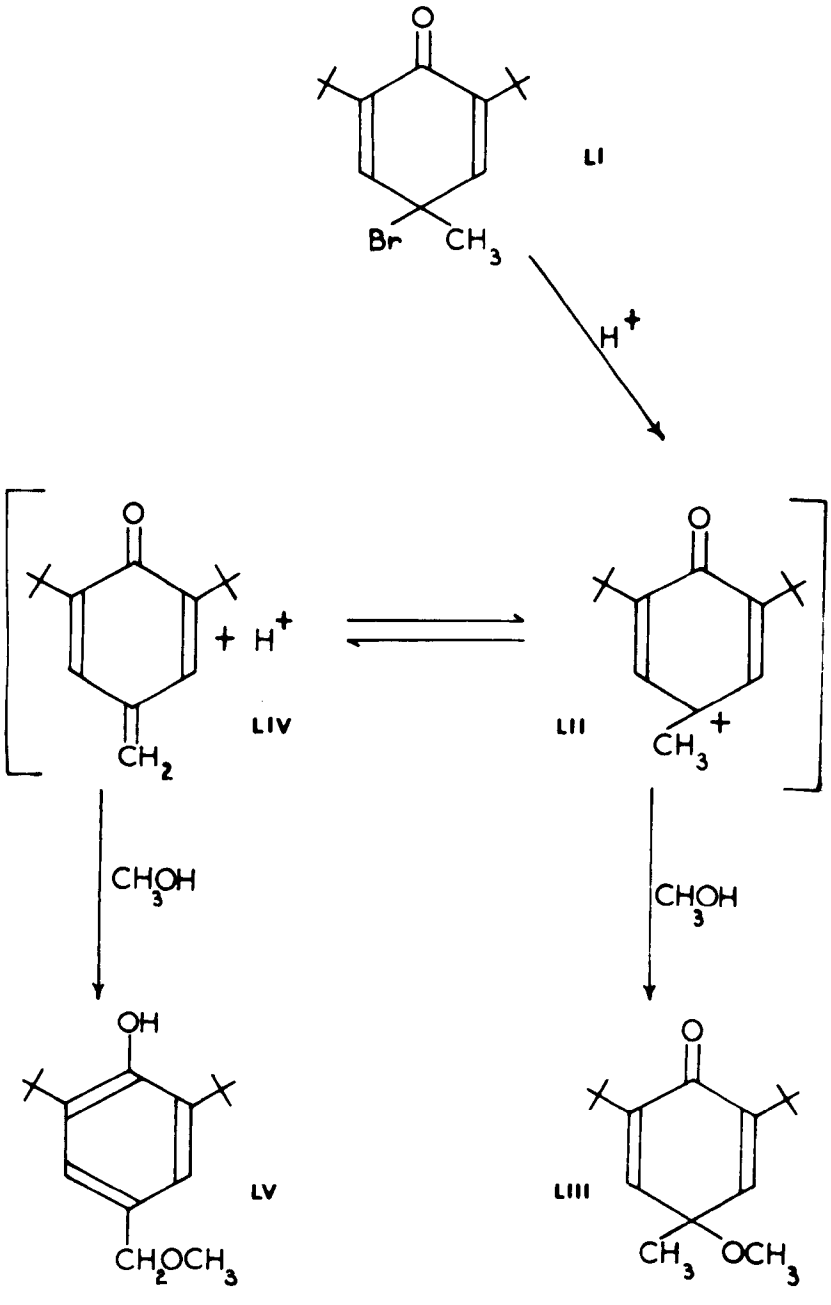

O
Br
CH3
LI
H+
+ H+
LIV
CH2
LII
CH3 +
CH3OH
CH3OH
OH
LV
CH2OCH3
LIII
O
CH3
OCH3

figure 28

As 3,5-di-t-nutyl-4-hydroxybenzyl acetate (LXV, R_1 = t-butyl, X = $CH_3C{:}O$-; figure 32) decomposes in acidic methanol to give 2,6-di-t-butyl-4-hydroxy-benzyl methyl ether (LXVII; R = t-butyl; figure 32) presumably via a quinone methide intermediate (LXVI; R = t-butyl; figure 32. Ref. 148), it is doubtful if a 4-hydroxy-benzyl phosphate would be produced under strongly acidic conditions.

2,6-di-t-Butyl-4-bromo-4-methyl-2,5-cyclohexadien-1-one, L1 (figure 28), has been reported to be quantitatively converted to 2,6-di-t-butyl-4-methoxy-4-methyl-2,5-cyclohexadien-1-one, LIII (figure 28), on boiling for a few minutes in methanol (102). In the present work this reaction was reinvestigated and the presence of a trace of acid was found to be a necessary requirement for the reaction to take place : when the bromo compound, L1, which had been dried over phosphorus pentoxide and sodium hydroxide, was dissolved in hot methanol and heated under reflux, no observable reaction occurred during half an hour. On adding a few drops of methanol containing a trace of glacial acetic acid, a rapid decolorisation of the yellow solution ensued. Recrystallisations of the crude product from polar and non-polar solvents gave a 64% yield of 2,6-di-t-butyl-4-methoxy-4-methyl-2,5-cyclohexadien-1-one (LIII, figure 28) and a 36% yield of 3,5-di-t-butyl-4-hydroxy-

benzyl methyl ether (LV, figure 28) respectively. The compounds were identified by comparison with authentic samples. The formation of these products can be rationalised by the reaction scheme shown in figure 28. Loss of bromide ion by an S_N1 process from the bromo compound, L1, results in the formation of the tertiary carbonium ion, LII, which can either react with methanol to form the 4-methoxy cyclohexadienone, LIII, or lose a proton from the 4-methyl group to form the quinone methide, LIV. Methanol can then react with this quinone methide in a 1, 6-addition reaction and form the 4-hydroxybenzyl methyl ether, LV. These observations suggest a possible synthesis of a 4-hydroxybenzyl phosphate using the bromo compound, L1, and disilver monophenyl phosphate in a non-aqueous solvent. Silver ions should facilitate the formation of the tertiary carbonium ion, LII, by electrophilic catalysis (149). Loss of a proton from the 4-methyl group of the tertiary carbonium ion, LII, would produce the quinone methide, LIV, which could react with the phosphate dianion in a nucleophilic 1, 6-addition reaction to produce 3, 5-di-t-butyl-4-hydroxybenzyl phosphate. Such a synthesis would have the advantages that the formation of the highly insoluble silver bromide favours the forward reaction and also the phosphate dianion is more nucleophilic than the less

basic phosphate monoanion or phosphoric acid used in the previous unsuccessful attempts to observe the addition of a phosphate to a quinone methide. 2,6-di-t-Butyl-4-bromo-4-methyl-2,5-cyclohexadien-1-one, L1, in dry acetonitrile reacted with disilver monophenyl phosphate to give several products and although paper chromatography showed monophenyl phosphate to be the only phosphorus-containing compound present in the reaction mixture, the various products could not be completely separated or identified. The reaction mixture was also examined directly by NMR spectroscopy but the spectrum obtained showed no characteristic doublet in the region 4 - 7 τ due to a benzyl $-CH_2-$ group split by coupling to phosphorus (141). Such a direct examination avoided any decomposition which may have resulted in a chromatography solvent or though an isolation procedure.

In this investigation of the reaction of phosphoric acid and its various anions with both stable and transient quinone methides, polymerisation of the quinone methide was the only reaction observed. No evidence for any nucleophilic addition of phosphate to a quinone methide was obtained.

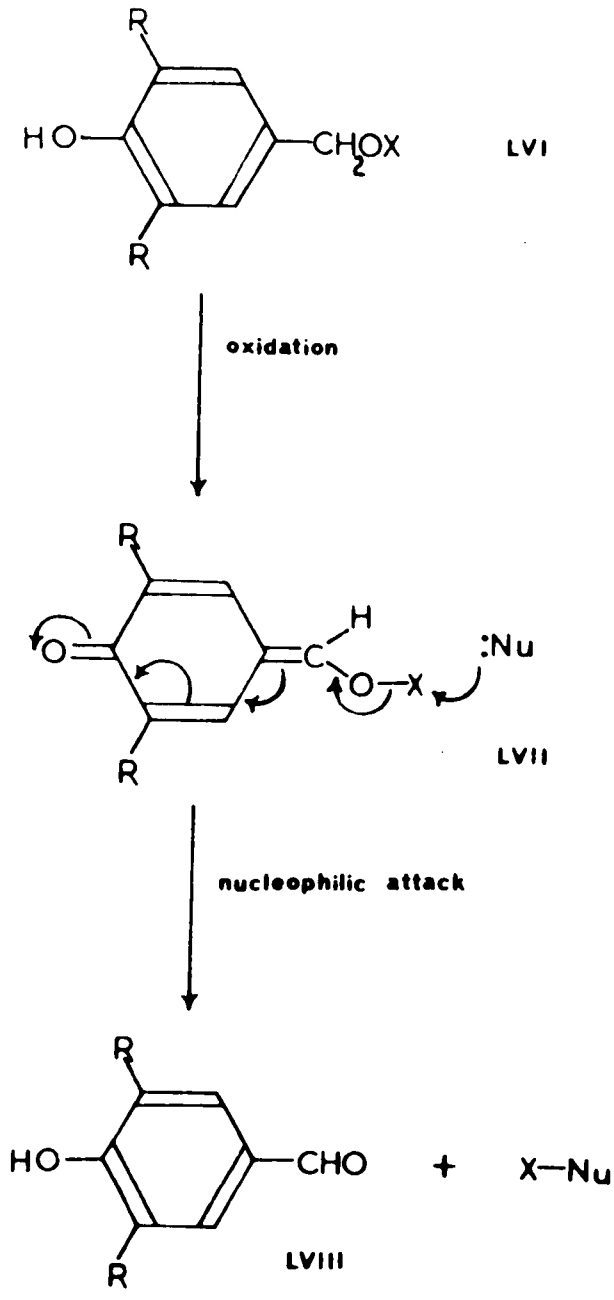

R
HO
CH2OX
LVI
R
oxidation
R
O
H
C
O
X
:Nu
LVII
R
nucleophilic attack
R
HO
CHO
+
X—Nu
LVIII
R

figure 29

THE ATTEMPTED SYNTHESIS AND OXIDATION OF SOME 4-HYDROXYBENZYL ESTER SYSTEMS

A synthesis of p-hydroxybenzyl phosphate and a study of its behaviour under oxidising conditions were attempted, with a view to obtaining evidence for steps 3 and 4 of the Erickson scheme for oxidative phosphorylation (figure 14).

Oxidation of a p-hydroxybenzyl phosphate, LVI (figure 29), for example by a suitable hydride ion abstractor such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (150), would give the quinone methide phosphate, LVII (figure 29), which is a P-XYZ system (52) and formally a vinylogous acid anhydride. For the P-XYZ system to be a good phosphorylating agent, Z must be able to accommodate the electrons of the P-X bond. Other parameters being equal, phosphorylation is facilitated when the P-X bond is weak. In the present case the phosphorus-oxygen $d\pi$-$p\pi$ bonding is attenuated by the dipolar character of the quinone methide which tends to direct the p electrons of the oxygen atom from $d\pi$-$p\pi$ interaction with the phosphorus atom into the quinone methide π electron system with resultant weakening of the phosphorus-oxygen ("P-X") bond. Attack by

a nucleophile at the phosphorus atom would lead to the terminal oxygen ("Z") formally accepting the electrons of the phosphorus-oxygen ("P-X") bond with aromatisation of the ring to give the p-hydroxybenzaldehyde, LVIII (figure 29). This aromatisation of the ring would further facilitate the phosphorylation reaction. Elimination of monomeric metaphosphate from the quinone methide phosphate, LVII (figure 29), could be an alternative mechanism.

An attempted synthesis of p-hydroxybenzyl phosphate LVI (R = H, X = P:O $(OH)_2$; figure 29) was undertaken from p-hydroxybenzaldehyde. In order to phosphorylate the benzylic hydroxyl group rather than the phenolic hydroxyl group, the latter had to be supplied with a protecting group. The benzyl group was inappropriate as benzyl esters cleave faster than benzyl ethers (151). The methoxymethyl group (152) was considered suitable since it could be cleaved under mildly acidic conditions which do not lead to the hydrolysis of a benzyl phosphate. If a phosphorochloridate were to be used as the phosphorylating agent, then it must bear protecting groups which could be removed under conditions which would not lead to the hydrolysis of benzyl phosphate. The 2, 2, 2-trichloroethyl group has been used as a protecting group in the synthesis of cephalosporin C (153) and

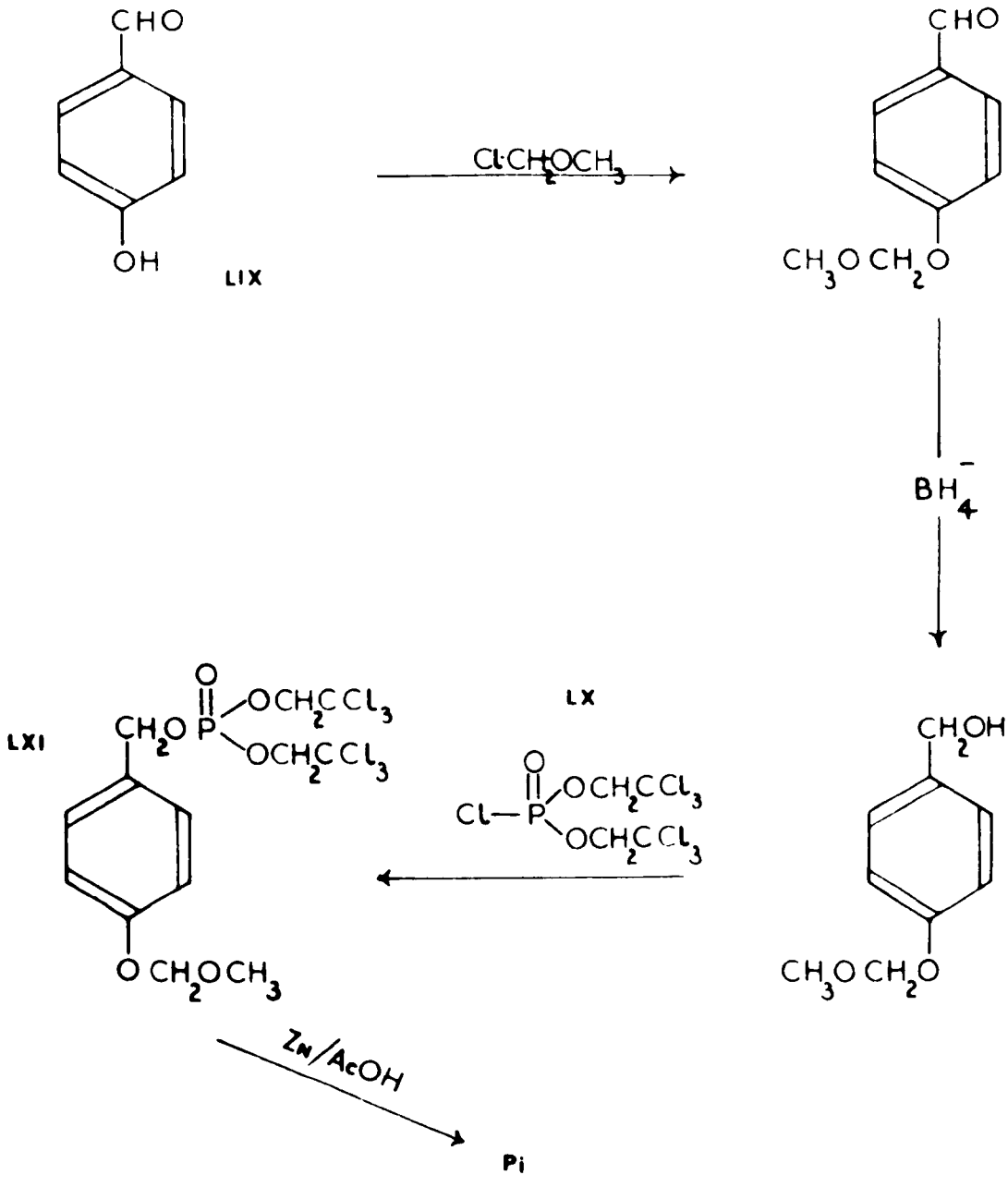

CHO
OH
LIX
Cl·CH2OCH3
CHO
CH3O CH2O
BH4
CH2OH
CH3O CH2O
LX
LXI
CH2O
OCH2CCl3
OCH2CCl3
Cl
OCH2CCl3
OCH2CCl3
O CH2OCH3
Zn/AcOH
Pi

figure 30

in the synthesis of nucleotides (154). It may be cleaved on treatment with zinc and 80% acetic acid, or other mild conditions, by an E2 elimination mechanism. The zinc attacks a chloro atom, the carbon-chlorine bond is broken, and phosphate anion is eliminated with formation of 2,2-dichloroethylene. The mechanism of this reaction suggests it would be specific for the cleavage of the 2,2,2-trichloroethyl group. For this reason bis-2,2,2-trichloroethyl phosphorochloridate (155) was chosen as the phosphorylating agent.

Treatment of p-hydroxybenzaldehyde, LIX (figure 30), with chloromethyl ether gave p-(methoxymethoxy)benzaldehyde, which on reduction with sodium borohydride yielded p-(methoxymethoxy)-benzyl alcohol. Phosphorylation of this alcohol with bis-2,2,2-trichloroethyl phosphorochloridate, LX (figure 30) gave bis-2,2,2-trichloroethyl 4-(methoxymethoxy)benzyl phosphate, LXI (figure 30), in essentially quantitative yield. The compound decomposed slowly on standing but was characterised spectroscopically. Treatment of this fully protected p-hydroxybenzyl phosphate, LXI, with zinc and 80% acetic acid or other reagents designed to remove the 2,2,2-trichloroethyl groups selectively resulted in the formation of inorganic phosphate as the major phosphorus-

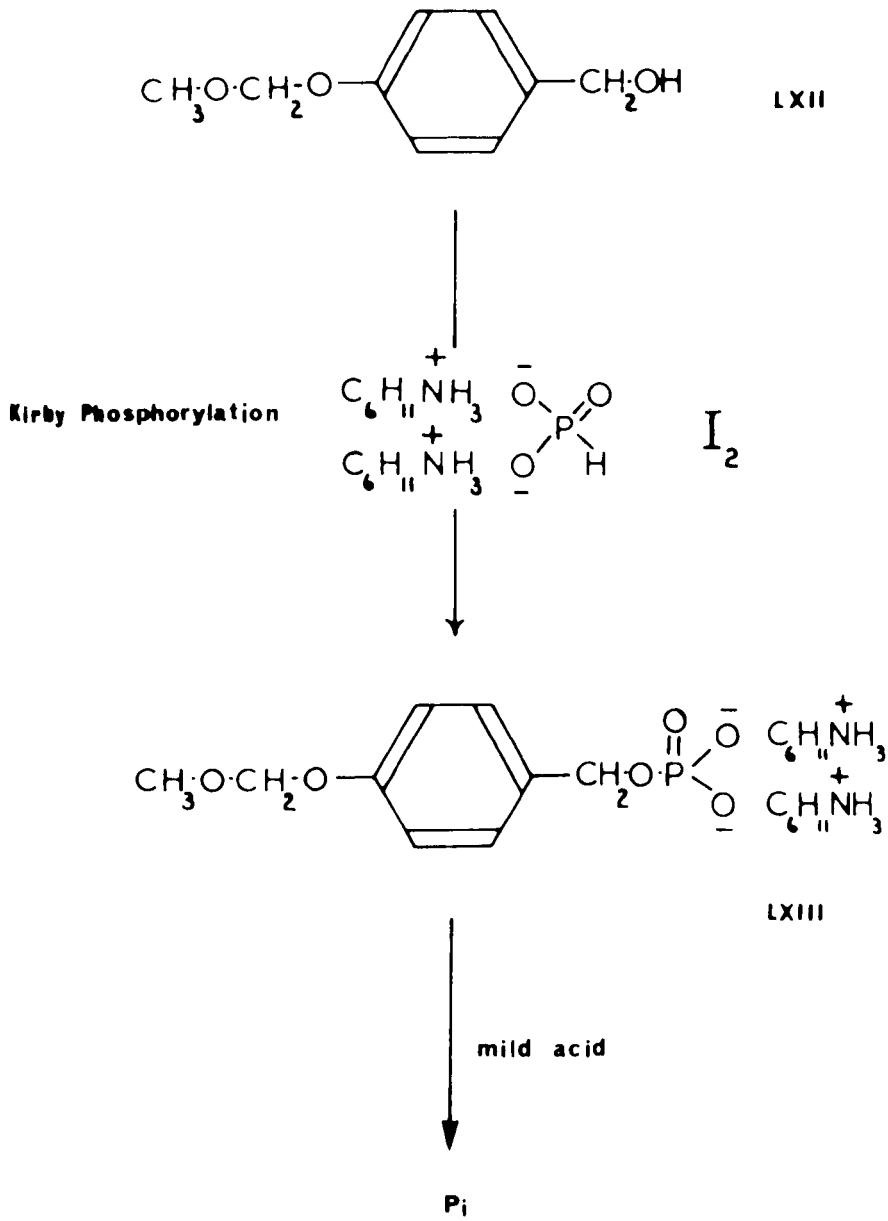

CH3·O·CH2·O
CH2·OH
LXII
Kirby Phosphorylation
C6H11NH3
C6H11NH3
I2
CH3·O·CH2·O
CH2·O·P
C6H11NH3
C6H11NH3
LXIII
mild acid
Pi

figure 31

containing compound. A small amount of phosphate monoester present in the reaction mixture was recovered in about 3% yield by anion-exchange chromatography and characterised as 2, 2-dichloroethyl phosphate. It presumably arose from 2, 2-dichloroethanol in the 2, 2, 2-trichloroethanol starting material. Indeed, analysis of the 2, 2, 2-trichloroethanol by vapour phase chromatography showed it to contain 3% 2, 2-dichloroethanol. The recovery of this impurity as its phosphate indicates that the above chromatographic isolation procedure would have detected even small quantities of p-hydroxybenzyl phosphate. A possible mechanism for the cleavage of the benzyl group of the phosphate triester would involve nucleophilic attack of chloride ion at the benzylic carbon atom with displacement of the phosphate diester monoanion. The cleavage of benzyl phosphate esters by anions is well known (156, 157) but unusual under such mild conditions.

4-(Methoxymethoxy)benzyl phosphate, LXIII (figure 31), was prepared from 4-(methoxymethoxy)benzyl alcohol, LXII (figure 31), by the method using iodine and the di-triethylammonium salt of phosphorous acid (158). A possible mechanism for this reaction is the initial formation of phosphoroiodate which spontaneously eliminates iodide anion to form monomeric metaphosphate,

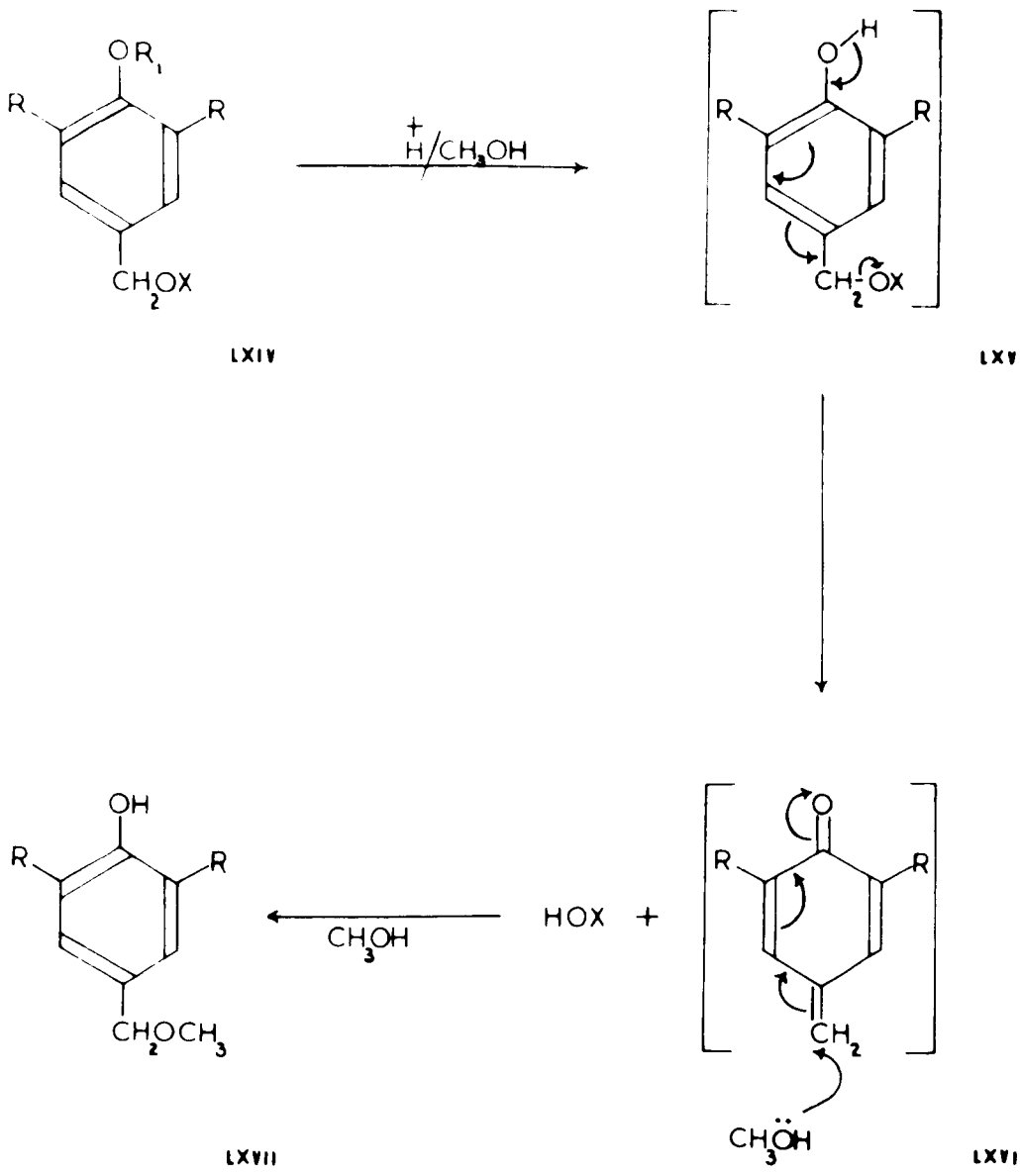

OR1
R
R
CH2OX
+
H/CH3OH
O
H
R
R
CH2OX
LXIV
LXV
OH
R
R
CH2OCH3
CH3OH
HOX +
O
R
R
CH2
CH3OH
LXVII
LXVI

figure 32

which then phosphorylates the alcohol. The 4-(methoxymethoxy) benzyl phosphate was completely characterised. The ester was examined by paper chromatography using isopropanol/ammonia/ water as solvent. On spraying the chromatogram with Hanes-Isherwood reagent (159) the ester appeared slowly at R_f 0.325 as a yellow spot characteristic of inorganic phosphate - hence the spray, which is highly acidic, cleaves the 4-(methoxymethoxy) benzyl phosphate to inorganic phosphate. When the methoxymethyl group was cleaved by warming a methanolic solution of the phosphate ester with a little Dowex 50W (H^+ form), inorganic phosphate was the only phosphorus-containing product. A possible mechanism for the decomposition is indicated in figure 32 (R = H, R_1 = CH_3OCH_2-, X = $P{:}O(OH)_2$-). The methoxymethyl group is cleaved under the acidic conditions to give 4-hydroxybenzyl phosphate, LXV, which immediately decomposes to inorganic phosphate and the quinone methide, LXVI. This then reacts with methanol to form 4-hydroxy-benzyl methyl ether. The reaction was carried out in ethanolic solution in the presence of a selection of oxidising agents to ascertain whether or not the transient p-hydroxybenzyl phosphate would be oxidised to the quinone methide phosphate, LVII (R = H, X = $P{:}O(OH_2)$-; figure 29), before non-oxidative decomposition to the quinone methide, LXVI (figure 32), and

inorganic phosphate occurred. The quinone methide phosphate, LVII, a P-XYZ system, would then phosphorylate ethanol to give ethyl phosphate. However, no ethyl phosphate could be detected by paper chromatography. The conclusion to be drawn is that the non-oxidative cleavage of p-hydroxybenzyl phosphate to inorganic phosphate and the quinone methide is very fast under acidic conditions.

The cleavage of the corresponding benzoate system was investigated. 4-(methoxymethoxy)benzyl benzoate was prepared from 4-(methoxymethoxy)benzyl alcohol and benzoyl chloride. The methoxymethyl group was cleaved by warming a methanolic solution of the ester in the presence of Dowex 50W resin (H^+ form), and resulted in a non-oxidative cleavage of the ester. The decomposition sequence is indicated in figure 32 (R = H, R_1 = CH_3OCH_2-, X = C_6H_5C:O-). Benzoic acid was recovered from this reaction in 99% yield and 4-hydroxybenzyl methyl ether, LXVII (R_1 = H; figure 32), in 92% yield. The reaction was carried out in ethanolic solution in the presence of a selection of oxidising agents to ascertain whether or not oxidative benzoylation could be observed with the transient p-hydroxybenzyl benzoate, before non-oxidative cleavage to benzoic acid and

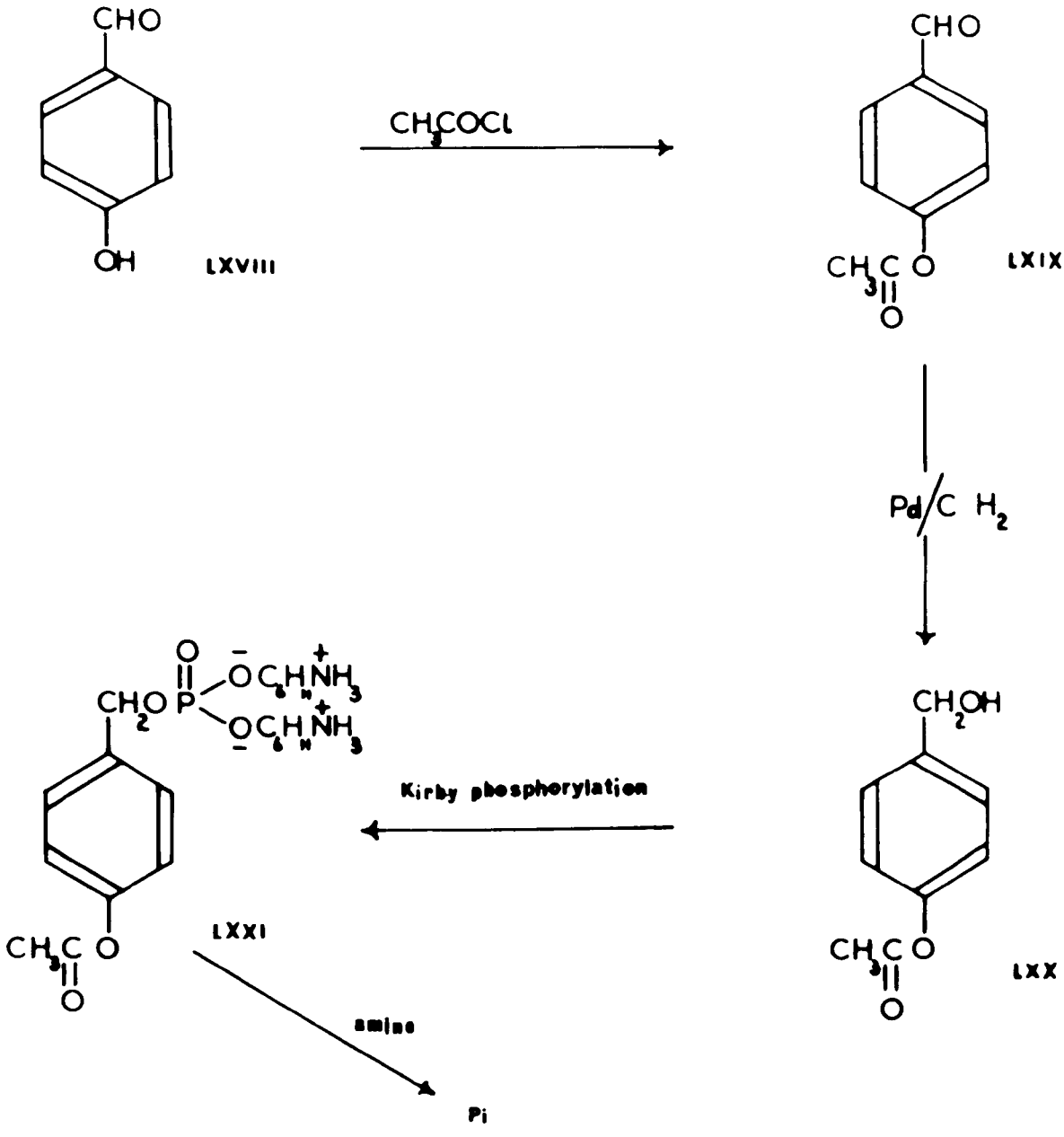

CHO
OH
LXVIII
CH3COCl
CHO
CH3C O
O
LXIX
Pd/C H2
CH2OH
CH3C O
O
LXX
Kirby phosphorylation
CH2O
P
O
OC6H11NH3
OC6H11NH3
CH3C O
O
LXXI
amine
Pi

figure 33

the quinone methide occurred. However, no ethyl benzoate could be detected although a gas chromatographic technique capable of detecting ethyl benzoate in 0.01% solution was used. The conclusion to be drawn is that the non-oxidative cleavage of 4-hydroxybenzyl benzoate to benzoic acid and the quinone methide is very fast under acidic conditions.

The facility of cleavage of the acetyl group under mildly basic rather than acidic conditions (152) is in contrast with the methoxymethyl group. For this reason a synthesis of 4-acetoxybenzyl phosphate (LXXI, figure 33) was undertaken. The sequence is indicated in figure 33. Treatment of p-hydroxybenzaldehyde, LXVIII, with acetyl chloride gave 4-acetoxybenzaldehyde, LXIX, which on reduction by hydrogenation in the presence of 10% palladium on charcoal gave 4-acetoxybenzyl alcohol, LXX. This was phosphorylated using phosphorous acid and iodine to give 4-acetoxybenzyl phosphate, LXXI, which was completely characterised. The phosphate ester was examined by paper chromatography and, while it was found to be unstable in a solvent of isopropanol/ammonia/water, in butanol/acetic acid/water it behaved as a phosphate monoester, $R_f = 0.75$. On spraying the chromatogram with Hanes-Isherwood reagent (159) the

ester appeared slowly as a yellow spot characteristic of inorganic phosphate. Hence the spray, which is highly acidic, cleaves the 4-acetoxybenzyl phosphate to inorganic phosphate. The acetyl group was cleaved by warming a solution of the 4-acetoxybenzyl phosphate in methanol containing a little cyclohexylamine. Inorganic phosphate was the only phosphorus-containing product, showing that any 4-hydroxybenzyl phosphate decomposed to inorganic phosphate and the quinone methide which then reacted with any nucleophile present in the reaction mixture. The reaction was repeated in ethanolic solution in the presence of a selection of oxidising agents to ascertain whether or not oxidative phosphorylation could be observed with any transient p-hydroxybenzyl phosphate; however, no ethyl phosphate could be detected by paper chromatography. It was concluded that the non-oxidative cleavage of 4-hydroxybenzyl phosphate to the quinone methide and inorganic phosphate is very fast under basic as well as acidic conditions.

4-Hydroxybenzyl phosphate may not be a true intermediate in the above reactions since the elimination of phosphate and formation of the quinone methide could be concerted with the cleavage of the methoxymethyl or acetyl group.

The facile cleavage of the trimethylsilyl protecting group

(160, 161) under neutral conditions contrasts with both the methoxymethyl and acetyl groups. A synthesis of 4-trimethylsiloxybenzyl phosphate was therefore undertaken, but failed - hydrogenation or borohydride reduction of 4-trimethylsiloxybenzaldehyde resulted in cleavage of the trimethylsilyl group.

4-Hydroxy-3,5-di-t-butylbenzyl acetate, LVI (R = t-butyl, X = $CH_3C{:}O$-; figure 29) provides an example of a compound of the type LVI which is stable and isolable. Its decomposition in acidic methanol follows the sequence shown in figure 32 (R = t-butyl, X = $CH_3C{:}O$-) to give 4-hydroxy-3,5-di-t-butylbenzyl methyl ether (148). Oxidation of the 4-hydroxybenzyl acetate could give a quinone methide acetate which is essentially a vinylogous acid anhydride and should be an acylating agent in accordance with the scheme shown in figure 29 (R = t-butyl, X = $CH_3C{:}O$-). To investigate if such oxidative acylation occurred with this compound, it was dissolved in ethanol and treated with a variety of oxidising agents, but no ethyl acetate was detected by a vapour phase chromatographic technique capable of detecting ethyl acetate in 0.01% solution.

EXPERIMENTAL SECTION

Abbreviations

The following abbreviations are used in the text.

M.Pt.	melting point
B.Pt.	boiling point
ν	wavenumbers of peaks in infra red spectrum
τ	tau values of peaks in NMR spectrum
gm.	gram
mgm.	milligram
M.	mole
mM.	millimole
conc.	concentrated
cts./s.	counts per second
VPC	vapour phase chromatography
TLC	thin layer chromatography
PPO	2,5 diphenyl oxazole
POPOP	1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene

UV Spectra

λ_{max}	maximum of absorbance in the UV region of the spectrum
mμ	millimicrons
ϵ	molar extinction coefficient at the wavelength previously indicated

UV Spectra

log.	logarithm to the base 10

IR Spectra

v.st.	very strong
st.	strong
med.st.	medium strong
w.	weak
v.w.	very weak
sh.	shoulder

NMR Spectra

s.	singlet
d.	doublet
t.	triplet
q.	quartet
c/s.	cycles per second
Mc/s.	megacycles per second

Solvents

Dioxan was heated under reflux, in an atmosphere of nitrogen, over sodium for 8 hours and then fractionally distilled. The initial distillate was discarded until the dioxan collected was spectroscopically pure. It was stored under nitrogen.

Methyl cyanide was heated under reflux over phosphorus pentoxide and then distilled. It was further purified by heating under reflux over potassium carbonate and finally fractionally distilled. B.Pt. 81 - 81.5^{o}C. It was stored over molecular sieve.

Other solvents were purified and dried by conventional means.

Chromatography

Vapour Phase Chromatography was carried out using a Perkin Elmer F11 instrument having a flame ionisation detector.

Ion-Exchange Chromatography was carried out using Dowex 1 (100 - 200 mesh, 8% cross linking) anion exchange resin using a technique of gradient elution with aqueous lithium chloride solution.

Thin Layer Chromatography was carried out with silica plates using 40:60 chloroform/benzene as eluent unless otherwise stated.

Paper Chromatography was carried out by the descending technique on Whatman No.4 paper. The following solvents were used.

A isopropanol/water/trichloracetic acid/conc. ammonia (75 mls. : 25 mls. : 5 gm. : 0.25 mls.)

B	n-butanol/acetic acid/water	(5 : 2 : 3)
C	isopropanol/conc. ammonia/water	(7 : 1 : 2)

Phosphates were detected by the method of Hanes and Isherwood (159). All paper chromatographic identifications were made by comparison with authentic samples.

Spectrometers

Ultra Violet spectra were recorded using a Carey 14 instrument.

Infra Red spectra were recorded using a Perkin Elmer 237 instrument.

NMR spectra were recorded at 60 Mc/s. using a Perkin Elmer R10 instrument.

Mass spectra were recorded using an A.E.I. M.S.9 mass spectrometer.

Melting Points are given uncorrected.

HYDROGEN ISOTOPE EXCHANGE IN METHYL QUINONES

PREPARATION OF STARTING MATERIALS

(1) Duroquinone was prepared from durene by the method described in Organic Syntheses (162).

M.Pt.	111 - 112°C (lit. (163) 111 - 112°C)
λ_{max} (dioxan)	257 mμ log ε = 4.26

(2) 2,3-Dimethyl-1,4-Naphthoquinone was prepared in 41% yield by a method analogous to that described for 1,4-naphthoquinone in Organic Syntheses (164).

M.Pt.	124 - 125°C (lit. (165) 127°C)
λ_{max} (dioxan)	246 mμ log ε = 4.28
	263 mμ log ε = 4.21

(3) Diduroquinone was prepared by the action of aqueous caustic soda solution on duroquinone in ethanol (94).

M.Pt.	202 - 203°C (lit. (166) 202 - 203°C)
ν (Nujol Mull)	3525 (v.st.), 1685 (v.st.) $cm.^{-1}$.
λ_{max} (dioxan)	254 mμ log ε = 4.10
	287 mμ log ε = 3.54

(4) The Dimer of 2,3-Dimethyl-1,4-Naphthoquinone was prepared by a modification of the method described by Chandrasenan and Thomson (167).

The reaction rate and purity of product were strongly dependent on the purity of the starting quinone. After purification by chromatography through an acid washed alumina column, 2,3-dimethyl-1,4-naphthoquinone (2.5 gm.) was dissolved in methanol and a few drops of 5N potassium hydroxide in methanol were added. After some time the solution turned red. After 12 hours the separated dimer was filtered off. The filtrate was poured into water and the crystals produced were filtered off. Recrystallisation of the crystals from ethanol yielded 1.5 gm. dimer (60%).

M.Pt. 226 - 228°C (lit. (168) 227 - 228°C)

(5) The p-Toluenesulphonate Ester of Diduroquinone (XLI). p-Toluenesulphonyl chloride (4 gm.) was added to diduroquinone (2 gm.) in pyridine (5 mls.). After being heated on a steam bath for 15 minutes, the mixture was poured into cold water and the crystals were filtered off. After washing with dilute hydrochloric acid, dilute caustic soda and water, they were recrystallised from ethanol to yield 1.6 gm. of the p-toluenesulphonate ester of diduroquinone (55% yield).

M.Pt. 197 - 198°C (Pale yellow crystals)

ν (Nujol Mull) 1700 (v.st.), 1680 (v.st.) $cm.^{-1}$

τ ($CDCl_3$)	2.42 (AB quartet $\Delta\nu_{AB}$ - 27.7 c/s., J_{AB} = 7.8 c/s)4H,
	6.9 - 7.2 (m.) 2H, 7.55 (s.) 3H,
	7.9 (s.), 8.0 (s.), 8.04 (s.), 8.12 (s.) 15H,
	8.58 (s.) 3H, 8.78 (s.) 3H.
λ_{max} (dioxan)	253 mμ log ε = 4.21
	225 mμ log ε = 4.48

Analysis :

Found	C, 67.18 ; H. 6.26 ; S, 6.56%
$C_{27}H_{30}O_6S$ requires	C, 67.19 ; H, 6.27 ; S, 6.64%

(6) The p-Toluene-sulphonate Ester of the Dimer of 2,3-Dimethyl-1, 4-Naphthoquinone (XLIII). was prepared in 78% yield by a method analogous to that described immediately above.

M.Pt.	198 - 199°C (white crystals)
ν (Nujol Mull)	1715 (v.st.), 1690 (v.st.) $cm.^{-1}$
τ ($CDCl_3$)	1.75 - 2.75 (m.) 12H, 7.26 (s.) 2H,
	7.55 (s.) 3H, 7.87 (s.) 3H,
	8.35 (s.) 3H, 8.56 (s.) 3H
λ_{max} (dioxan)	299 mμ, 264 mμ
	226 mμ log ε = 4.88

Analysis :

Found	C, 70.56 ; H, 5.15 ; S, 5.86%
$C_{31}H_{26}O_6S$ requires	C, 70.68 ; H, 4.98 ; S, 6.08%

HYDROGEN ISOTOPE EXCHANGE IN METHYL QUINONES

(A) Studies using Infra Red Spectroscopy

(1) 2,3-Dimethyl-5,6-Bispiperidinomethylquinol (90). Duroquinone (2.5 mM.) was dissolved in piperidine (20 mls.) to give a 0.125 M solution which was left for 36 hours at room temperature. The solvent was removed on the rotary evaporator and the residue recrystallised three times from ethanol to give 2,3-dimethyl-5,6-bispiperidinomethylquinol (0.6 mM 24% yield). The solution was monitored by TLC on silica plates at suitable intervals. The duroquinone had essentially disappeared after 7 hours.

M.Pt.	161 - 162°C (lit. (90) 161 - 162°C)
ν (HCB Mull)	3300 - 2300 (med. v. broad)
λ_{max} (ethanol)	304, 298 mμ

(2) 2,3-Dimethyl-5,6-Bismorpholinomethylquinol (90). Duroquinone (4.8 mM.) was dissolved in morpholine (40 mls.) to give a 0.12 M solution which was left for 72 hours at room temperature. The solvent was removed on the rotary evaporator and the residue recrystallised from ethanol to give 2,3-dimethyl-5,6-bismorpholinomethylquinol (1.5 mM., 31% yield). The solution was monitored by TLC on silica plates at suitable intervals. The duroquinone had essentially disappeared

after 70 hours.

M.Pt.	215 - 217°C (decomp.) (lit. (90) 208 - 209°C (decomp.))
ν (HCB Mull)	3400 - 2400 (med. v. broad), 2970 (st.), 2930 (st.), 2870 (st.), 2830 (st.)
λ_{max} (ethanol)	304, 298 mμ

(3) 2,5-Dimethyl-5,6-Bispyrrolidinomethylquinol. Duroquinone (1.2 mM.) was dissolved in pyrrolidine (10 mls.) to give a 0.12M solution which was left for 3 hours at room temperature. The solvent was removed on the rotary evaporator and the residue recrystallised from ethanol. Brown crystals were obtained and these seemed to decompose completely after a few hours. Spectral data was consistent with the structure of 2,5-dimethyl-5,6-bis-pyrrolidinomethylquinol.

M.Pt.	131°C (darkening 120°C approx.)
ν (HCB Mull)	3400 - 2100 (med. v. broad) $cm.^{-1}$
λ_{max} (ethanol)	304, 296 mμ

(4) Preparation of N-Deuteromorpholine. A mixture of deuterium oxide (75 mls.) and morpholine (75 mls.) was fractionally distilled. The third fraction was collected (B.Pt. 128°C).

ν (Thin Film)	3300 (st. broad), 2490 (st. broad)

(5) The Attempted Deuteration of Duroquinone with N-Deuteromorpholine

Duroquinone (0.1 gm.) was dissolved in N-deuteromorpholine (5 mls.) and left for 48 hours at room temperature. The solvent was removed on the rotary evaporator and the residue recrystallised from ethanol to yield 2,3-dimethyl-5,6-bismorpholinomethylquinol identical (including IR spectrum) with that prepared from undeuterated morpholine. The mother liquors were evaporated to small bulk and the residue separated into its components by TLC. The recovered duroquinone was identical with the starting material (including IR spectrum). No observable deuteration of the quinone or the product had occurred.

(6) Observation of Hydrogen/Deuterium exchange in Duroquinone.

Potassium bicarbonate (0.5 gm.) was added to duroquinone (300 mgm.) in deuterium oxide (7 mls.) and dioxan (12 mls.). The mixture was heated under reflux (mercury seal) for 48 hours, cooled, acidified with dilute hydrochloric acid and extracted with ether. The ethereal extract was dried over magnesium sulphate, evaporated to small bulk and separated into its components by TLC. Duroquinone was recovered in about 1% yield, diduroquinone in more than 90% yield.

ν (HCB Mull) (duroquinone)	3270 (med.), 3030 (med.), 2960 (med.st.), 2940 (med.st.), 2870 (med.), 2250 (med.), 2210 (med.) 2130 (w.), 2060 (w.), 1640 (v.st.) $cm.^{-1}$

ν (HCB Mull)	3530 (v.st.), 3000(st.), 2980 (st.), 2930 (v.st.),
(diduroquinone)	2240 (w.), 2200 (w.), 2110 (w.), 2060 (w.)
	1680 (v.st.) cm.$^{-1}$.

Underlining indicates peaks not present in the undeuterated forms. The experiment was repeated using triethylamine (1 ml.) in place of potassium bicarbonate with similar results.

(7) Observation of Hydrogen/Deuterium exchange in 2,3-Dimethyl-1,4-Naphthoquinone. 2,3-dimethyl-1,4-naphthoquinone (150 mgm.) was added to triethylamine (0.25 mls). in deuterium oxide (1.5 mls.) and dioxan (3.25 mls.). The mixture was heated under reflux (mercury seal) for 10 hours, cooled and acidified with dilute hydrochloric acid and extracted with ether. The ethereal solution was dried over magnesium sulphate, evaporated to small bulk and separated into its components by TLC. 2,3-Dimethyl-1,4-naphthoquinone was recovered in about 30% yield.

ν (HCB Mull)	3300 (v.w.), 3100 (v.w.), 3080 (v.w.), 2960 (w.),
	2930 (w.), 2860 (v.w.), 2260 (v.w.), 2220 (v.w.)
	1660 (v.st.) cm.$^{-1}$

Underlining indicates peaks not present in the undeuterated forms.

(b) Studies using a Liquid Scintillation Spectrometer

(1) The Stability of Diduroquinone Under the Exchange Conditions. Diduroquinone (200 mgm.) was dissolved in dioxan (5 mls.) and water (2.5 mls.). Triethylamine (0.5 mls.) was added and the mixture heated under reflux for 48 hours. The mixture was cooled, acidified and extracted with ether. The ethereal extract was examined by TLC and found to contain a trace of duroquinone, mostly unchanged diduroquinone and some material which remained at the origin of the thin layer chromatogram.

(2) The Stability of the Tosyl Ester of Diduroquinone under the exchange conditions. The previous experiment was repeated using the tosyl ester of diduroquinone in place of diduroquinone. TLC of the ethereal extract showed it to contain mainly unchanged starting material together with a little material which remained at the origin of the thin layer chromatogram.

(3) The Stability of the Dimer of 2, 3-Dimethyl-1, 4-Naphthoquinone under the Exchange Conditions. The dimer of 2, 3-dimethyl-1, 4-naphthoquinone (200 mgm.) was dissolved in dioxan (5 mls.) and water (2.5 mls.). Triethylamine (0.5 mls.) was added and the mixture heated under reflux for 10 hours. The mixture was cooled, acidified and extracted with ether. The ethereal extract was examined by TLC and found to contain mainly monomeric 2, 3-dimethyl-1, 4-naphthoquinone together

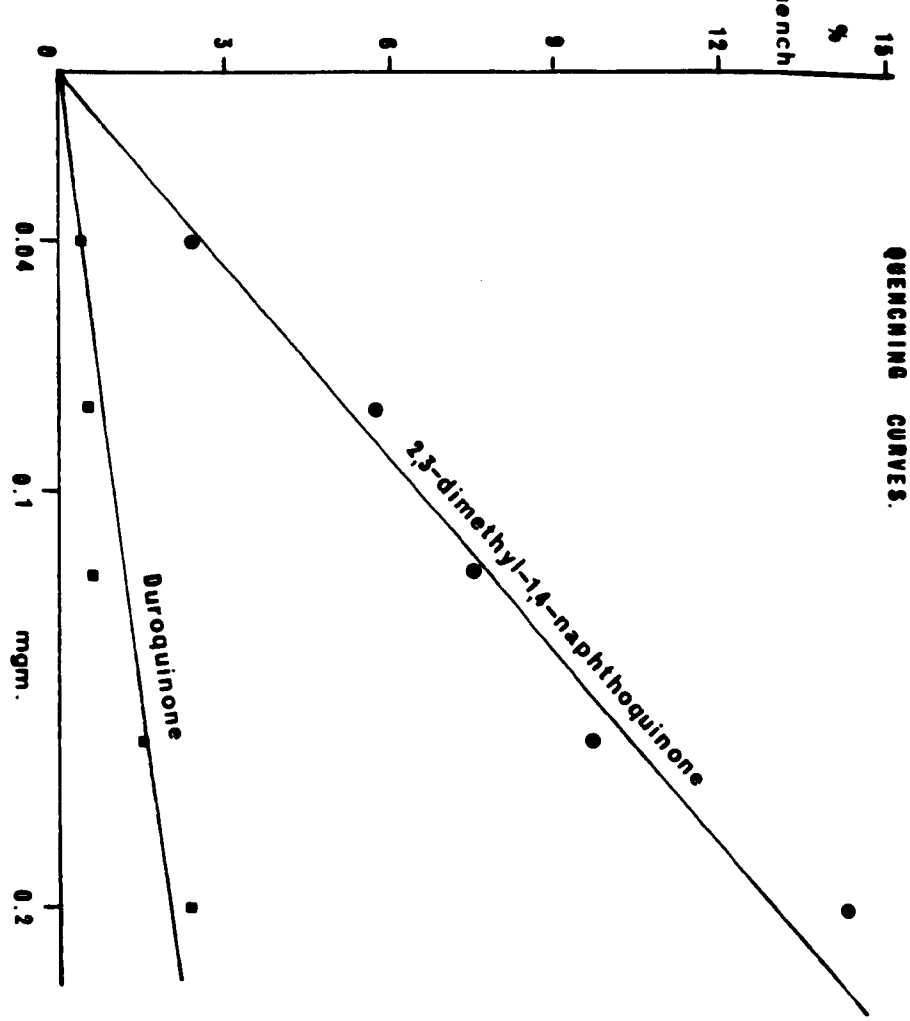

QUENCHING CURVES.
% quench
15
12
9
6
3
0
0.04
0.1
0.2
mgm.
2,3-dimethyl-1,4-naphthoquinone
Buroquinone

figure 34

with some material which remained at the origin of the thin layer chromatogram. No dimer was detected.

(4) The Stability of the Tosyl Ester of the Dimer of 2,3-Dimethyl-1,4-Naphthoquinone Under the Exchange Conditions. The previous experiment was repeated using the tosyl ester derivative in place of dimer. The ethereal extract was examined by TLC and found to contain mainly unchanged tosyl ester derivative together with a little material which remained at the origin of the thin layer chromatogram.

(5) The Quenching Effect of Quinones and Derivatives on the scintillation counts observed in the liquid scintillation spectrometer. Duroquinone solution (5 mls.) of suitable concentration was added to tritium containing scintillation fluid (5 mls. of a stock solution containing 8 gm. PPO, and 0.4 gm. POPOP per litre, together with a suitable quantity of tritiated water) in a Packard Tri-Carb vial and left for a few hours in the dark in order to reach equilibrium. The actual counts in each of a series of vials containing different quantities of duroquinone were measured using a Packard Tri-Carb liquid scintillation spectrometer. The experiment was repeated using 2,3-Dimethyl-1,4-naphthoquinone in place of duroquinone. The results are indicated in figure 34. Diduroquinone, the tosyl ester of diduroquinone and the tosyl ester of the dimer of 2,3-dimethyl-1,4-naphthoquinone were without a significant quenching effect at the concentrations used.

(6) The Hydrogen Isotope Exchange of Duroquinone and its Derivatives.

Duroquinone (300 mgm.) was dissolved in a solution containing dioxan (40 mls.), water (8 mls.), triethylamine (5 mls.) and tritiated water (2 mls. Total cts./s. = 6.791×10^8). Sealed ampoules each containing 5 mls. of the stock solution were heated in an oven at $103^{\circ}C$ and were removed singly with a suitable time interval between samples. Each sample was cooled, acidified with dilute hydrochloric acid and extracted with ether. The ether extract was dried over magnesium sulphate and evaporated to dryness. The residue was extracted with carbon tetrachloride (not all of the residue dissolved) and the solution separated into its components by preparative TLC. The duroquinone band was recovered from the plate and run on a second and then a third TLC plate for complete purification. The duroquinone was extracted from the final plate using spectroscopically pure dioxan. After careful filtration, this solution (or a portion of it) was diluted to a concentration of approximately 0.02 mgm./ml. The exact concentration was determined by ultra violet spectroscopy. This duroquinone solution (5 mls.) was added to scintillation counting fluid (5 mls. of a stock solution containing 8 gm. PPO and 0.4 gm. POPOP per litre) in a Packard Tri-Carb vial which was then left in the dark for a few hours in order to reach equilibrium. The tritium content of the solution was

measured using a Packard Tri-Carb liquid scintillation spectrometer. The observed counts were corrected for background radiation and the quenching effect of the duroquinone. If the recovered quinone gave x counts/second/mole of exchangeable hydrogen and the water of the stock solution gave y counts/second/mole of exchangeable hydrogen then the scrambling was $\frac{x}{y}$ x 100%. The results are indicated in figure 19. Simultaneous experiments were carried out using diduroquinone and the tosyl ester of diduroquinone in place of duroquinone. The results are indicated in figure 19.

(7) The Hydrogen Isotope Exchange of 2,3-Dimethyl-1,4-Naphthoquinone and its Derivatives. The previous experiment was repeated using a stock solution containing 2,3-dimethyl-1,4-naphthoquinone (300 mgm.), dioxan (35 mls.), triethylamine (2.5 mls.), water (10 mls.) and tritiated water (2.5 mls., total cts./s. = 7.2604×10^9) at temperatures of 103°C, 60°C and room temperature. The results are indicated in figure 20. The experiment at 103°C was repeated using the tosyl ester of the dimer of 2,3-dimethyl-1,4-naphthoquinone in place of the momeric quinone. Essentially the ester did not exchange.

(8) The Hydrogen isotope Exchange of 2,3-Dimethyl-1,4-Naphthoquinone at various pH's. A buffer system based on Britton-Robinson buffers (140). was made up in dioxan/water.

Solution A — Acetic Acid (0.6 gm.), phosphoric acid (1 gm.), Boric Acid (0.6 gm.) in 2:1 dioxan/water (250 mls.).

Solution B — Sodium hydroxide (2 gm.) in 2:1 dioxan/water (250 mls.)

Various buffers were made up from the stock solutions and the "pH" measured using a glass electrode pH meter. A series of sealed ampoules each containing buffer (5 mls.), tritiated water (0.5 mls.) and 2,3-dimethyl-1, 4-naphthoquinone (30 mgm.) were heated at 96°C for 4 hours. A further ampoule contained IN caustic soda in 2:1 dioxan/water (5 mls.) in place of buffer solution. For each sample the quinone was recovered and its tritium content determined using the usual procedure. The results are indicated in figure 21.

(9) 2-Methyl-3-(3,7,11,15-tetramethylhexadecyl)-1,4-Naphthoquinone (169) hereafter referred to as perhydrovitamin K_1. Adam's catalyst (200 mgm.) was added to vitamin K_1 (4 gm.) in ethanol (150 mls.). The mixture was hydrogenated at 1 atmosphere pressure. After the theoretical quantity of hydrogen had been consumed, the catalyst was filtered off and the filtrate shaken overnight with freshly prepared silver oxide. The mixture was filtered and the filtrate evaporated to dryness. The remaining oil was purified by chromatography through a column of Decalso F silica using 40/60 petreoleum ether as eluent.

The yellow solution collected was evaporated to dryness to give perhydro-vitamin K_1 (1.5 gm., 38% yield) as a yellow oil.

ν (CCl_4)	1.95 - 2.1 (complex m.), 2.3 - 2.45 (complex m.), 7.4 (t., J = 8.4 c/s.), 7.9 (s.), 8.75 (broad s.), 9.1 (s.), 9.2 (s.).
λ_{max} (dioxan)	243 (sh.), 248, 261, 272 (sh.) mμ

Compare vitamin K_1

ν (CCl_4)	1.85 - 2.1 (complex m.), 2.25 - 2.45 (complex m.), 5.0 (t., J = 6 c/s.), 6.7 (d., J = 6 c/s.), 7.85 (s.), 8.2 (t.), 8.8 (broad s.), 9.1 (s.), 9.2 (s.).
λ_{max} (dioxan)	233, 243 (sh.), 272, 282 mμ

(10) The Stability of Vitamin K_1 and Perhydro Vitamin K_1 under the Exchange Conditions. A sealed ampoule containing vitamin K_1 (18.9 mgm.), dioxan (4 mls.), water (1 ml.), triethylamine (0.6 mls.) was heated for 4 hours at 100°C. No vitamin K_1 could be recovered by the usual procedure. The experiment was repeated using perhydrovitamin K_1 in place of vitamin K_1. Some perhydrovitamin K_1 could be recovered by the usual procedure. Both experiments were repeated at room temperature with similar results.

(11) The Hydrogen Isotope Exchange of Perhydrovitamin K_1. Sealed ampoules each containing 5 mls. of a stock solution containing perhydrovitamin K_1 (396 mgm.), dioxan (40 mls.), water (8 mls.), triethylamine (6 mls.) and tritiated water (2 mls., Total c/s. = 6.8032×10^9) were heated at 103°C for varying lengths of time. In each case the perhydrovitamin K_1 was recovered and its tritium content measured by the normal procedure. The results are indicated in figure 22.

(C) Studies using Mass Spectroscopy

(1) Hydrogen Isotope Exchange of 2,3-Dimethyl-1,4-Naphthoquinone. Sealed ampoules, each containing 5 mls. of a stock solution of 2,3-dimethyl-1,4-naphthoquinone (1.5 gm.), dioxan (32.5 mls.), deuterium oxide (15 mls.), and triethylamine (2.5 mls.), were heated at 103°C for varying lengths of time. In each case the quinone was recovered by the usual procedure. The deuterium content of the quinone was measured using mass spectroscopy. For each quinone sample repeated scans of the parent group were taken at 5 minute intervals (3 in all). By averaging the peak intensity for each peak at a particular m/e, any error due to alterations in source intensity was eliminated. To allow for the natural spread of peaks the following simple correction was applied to all spectra of the deuterated samples. If I_m represents the observed intensity of a peak at m/e = M, then the corrected intensity of that peak, I_m^c, was given by

$$I^{c}_{m} = I_{m} - I_{m-1} \times \theta$$

where I_{m-1} is the observed intensity of the peak corresponding to m/e = M - 1 and θ is $\frac{I_{187}}{I_{186}}$ for the undeuterated sample. Using the corrected peak intensities the deuterium content of the quinone was calculated. The experiment was repeated at 60°C. The results are indicated in figure 24. The groups of parent ions of the mass spectra of deuterated and non-deuterated 2,3-dimethyl-1,4-naphthoquinone are shown in figure 23(b).

(2) The Combined Primary and Solvent Isotope Effect. Sealed ampoules, each containing 4 mls. of a stock solution of 2,3-dimethyl-1,4-naphthoquinone (200 mgm.), deuterium oxide (6 mls.), dioxan (18 mls.) and triethylamine (1.25 mls.), were heated at 46°C for varying lengths of time. In each case the quinone was recovered and its deuterium content measured by the normal procedure. A simultaneous experiment using protium oxide in place of deuterium oxide and 2,3-di-perdeuteromethyl-1,4-naphthoquinone (95% deuterated) in place of 2,3-dimethyl-1,4-naphthoquinone was carried out. The results of the two simultaneous experiments are indicated in figure 25.

(D) Studies using NMR Spectroscopy

The NMR spectra of the 2,3-dimethyl naphthoquinone samples recovered in the experiments described above were recorded and the deuterium content of the quinone calculated from the observed peak intensities. The

The four aromatic hydrogens of the quinone served as an internal reference in each sample. The measured deuterium content agreed to within 5% of the value determined by mass spectroscopy. The NMR spectra of the deuterated and non-deuterated, 2,3-dimethyl-1,4-naphthoquinone are indicated in figure 23 (a).

THE ATTEMPTED ADDITION OF PHOSPHATE TO A QUINONE METHIDE

PREPARATION OF STARTING MATERIALS

(1) Tetra-n-Butyl-Ammonium Phosphates were prepared from solutions of the acid and tetra-n-butyl ammonium hydroxide after preliminary titration. Water was removed by freeze drying.

(2) 2,5-Di-t-Butyl-4-Isopropyl Phenol was prepared in 82% yield by the action of isobutylene on 4-isopropyl phenol (99).

M.Pt. 38 - 42°C (lit. (99) 38 - 42°C)

τ (CCl_4) 3.06 (s.) 2H, 5.2 (br.s.) 1H, 7.22 (m) 1H, 8.5 (s.) 18H, 8.80 (d. J = 6.6 c/s) 6H.

(3) 2,6-Di-t-Butyl-4-Isopropylidene-2,5-Cyclohexadien-1-One was prepared in 50% yield by oxidation of the parent phenol with alkaline ferricyanide solution (99). The product was purified by chromatography of the crude product on a column of Decalso F silica using 1% ether in petroleum ether as eluent.

M.Pt. 103.5 - 105.5°C (lit. (99) 103.5 - 105.5°C)

τ (CCl_4) 2.51 (s.) 2H, 7.73 (s.) 6H, 8.63 (s.) 18H.

(4) 2,6-Di-t-Butyl-4-Ethyl Phenol was prepared in 71% yield by the reaction of 4-ethyl phenol with isobutylene (170).

M.Pt. 44°C (lit. (170) 44°C)

τ (CCl_4) 3.1 (s.) 2H, 5.18 (s.) 1H,

7.49 (q. J = 7.8 c/s) 2H, 8.65 (s.) 18H,

8.82 (t. J = 7.8 c/s) 3H

(5) 2,6-Di-t-Butyl-4-Ethylidene-2,5-Cyclohexadien-1-One was prepared in 60% yield by the oxidation of the parent phenol with alkaline ferricyanide (171).

M.Pt. 89 - 91°C (lit. (171) 92.0 - 93.5°C)

τ (CCl_4) 3.00 (AB quartet, $\Delta\nu_{AB}$ = 28.6 c/s, J = 3.6 c/s) 2H,

3.72 (q., J = 7.8 c/s) 1H,

7.91 (d., J = 7.8 c/s) 3H, 8.7 (s.) 18H.

(6) 2-Methyl-1,4-Naphthoquinone was prepared in 33% yield by the oxidation of 2-methyl naphthalene with chromic oxide, analogous to the method described for 1,4-naphthoquinone in Organic Syntheses (164).

M.Pt. 104 - 105°C (lit. (172) 104°C)

(7) Diazomethane in ethereal solution was prepared from N-methyl nitrosourea as described by Vogel (173).

(8) 3a,4,9,9a-Tetrahydro-9a-Methyl-4,9-Dioxo-3H-Benz(f)indazole was prepared in 57% yield by the reaction of diazomethane with 2-methyl-1,4-naphthoquinone (174).

M.Pt. 111 - 112°C (decomp.) (lit. (168) 111°C, (lit. (174) 114°C)

(9) 3,3'-Dimethyl-2,2'-Ethylene-Di-1,4-Naphthoquinone was prepared in 86% yield by the action of aqueous alkali on the above indazole (174).

M.Pt.	268 - 270°C (lit. (174) 278°C lit. (175) 269 - 270°C)
ν (Nujol Mull)	1658, 1610, 1595, 795, 712, 686 (all st.) cm^{-1}.
τ ($CDCl_3$)	1.7 - 2.3 (m.) 8H, 7.12 (s.) 4H, 7.65 (s.) 6H.

(10) 2,6-Di-t-Butyl-4-Bromo-4-Methyl-2,5-Cyclohexadien-1-One was prepared by the reaction of the parent phenol with bromine (102). 2,6-di-t-Butyl-4-methyl-phenol (5 gm.) was dissolved in glacial acetic acid (67 mls.) and water (5 mls.) added. Bromine (1.15 mls.) was added dropwise with shaking. After a few seconds the yellow precipitate was filtered off and washed with a little glacial acetic acid and then recrystallised from petroleum ether (60 - 80°C) and dried over phosphorus pentoxide and sodium hydroxide . (57% yield).

M.Pt.	91 - 91.5°C (lit. (102) 91 - 91.3°C)
ν (Nujol Mull)	1660, 1640 (v.st.d.) cm^{-1}.
τ (CCl_4)	3.22 (s.) 2H, 8.05 (s.) 3H, 8.76 (s.) 18H

(11) Disilver Monophenyl Phosphate was obtained by mixing solutions of disodium monophenyl phosphate and silver nitrate (176).

Analysis : Found Ag, 54.2% Calculated for $C_6H_5Ag_2O_4P$; Ag, 55.7%

(12) 3,5-Di-t-Butyl-4-Hydroxybenzyl-N,N-Diethyl-Dithiocarbamate was prepared in 33% yield by the method patented by Canadian Ind. Ltd. (177).

M.Pt. 97 - 99°C (lit. (177) 98°C)

(13) 2,6-Di-t-Butyl-4-Hydroxybenzyl Methyl Ether. The above diothiocarbamate (1 gm.) was dissolved in methanol (10 mls.) and a few drops of 2N potassium hydroxide in methanol were added, and the solution left overnight at room temperature. The solution was diluted with water, acidified with dilute hydrochloric acid, and extracted with ether. The ether extract was dried and the ether removed by evaporation. The residue was recrystallised from ethanol/water.

M.Pt. 98 - 99°C (lit. (178) 98°C) 80% yield

ν (Nujol Mull) 3540 (v.st.br.) cm^{-1}.

τ (CCl_4) 3.06 (s.) 2H, 5.05 (s.) 1H, 5.80 (s.) 2H, 6.75 (s.) 3H, 8.56 (s.) 18H.

THE ATTEMPTED ADDITION OF PHOSPHATE TO A QUINONE METHIDE

(A) The Reaction of Duroquinone with Inorganic Phosphate in Non-aqueous Solvents. Tetra-n-butyl-ammonium dihydrogen phosphate (0.34 mM.) was added to duroquinone (1.83 mM.) in dioxan (10 mls.) and methanol (2 mls.) and the mixture was heated under reflux for 15 hours. No reaction occurred and unchanged duroquinone could be recovered by TLC. The experiment was repeated using di-tetra-n-butyl-ammonium monohydrogen phosphate (0.52 mM). Purification by TLC of the black solution which was formed yielded a black compound (R_f = 0) and an orange compound (R_f = 0.5) which was characterised as diduroquinone (M.Pt. and mixed M.Pt. 202°C. ν (Nujol Mull) 3525 (v.st.), 1685 (v.st.) cm^{-1}). The experiment was repeated using tri-tetra-n-butyl-ammonium phosphate (0.37 mM.) with identical results to the dianion case. All three experiments were repeated using an excess of phosphate and all six experiments were repeated at room temperature with identical results. Paper chromatography (solvents B and C) of all the final reaction mixtures showed inorganic phosphate as the only phosphorus-containing compound.

(B) The Attempted Direct Addition of Monophenyl Dihydrogen Phosphate to a Stable Quinone Methide. These reactions were carried out using a modification of the method described by Thompson (107). 2,6-di-t-Butyl-4-isopropylidene-2,5-cyclohexadien-1-one (0.25 mM.) was dissolved

in hexane (10 mls.) and a solution of monophenyl dihydrogen phosphate (0.25 mM.) in dioxan (5 mls.) was added. The resulting solution became colourless on being saturated with gaseous anhydrous hydrogen chloride and after one hour at room temperature the solution was heated on a steam bath for 5 hours and then left overnight at room temperature. Paper chromatography (solvents B and C) showed monophenyl phosphate as the only phosphorus-containing compound. The reaction mixture was evaporated to dryness and chloroform (10 mls.) added, and the resulting solution extracted twice with water. Paper chromatography (solvents B and C) of the aqueous layer showed MPP as the only phosphorus-containing compound and of the non-aqueous layer showed no phosphorus-containing compounds. TLC of the chloroform layer showed a large number of products which were not identified. The experiment was repeated using 2,6-di-t-butyl-4-ethylidene-2,5-cyclohexadien-1-one (0.25 mM.) with similar results. Both experiments were repeated with the omission of hydrogen chloride with similar results.

(c) The Attempted Addition of Phosphate to the 3-Methyl-1, 4-Naphthoquinone-2-Ylmethyl Carbanion produced from 3a,4,9,9a-Tetrahydro-9a-Methyl-4,9-Dioxo-3H-Benz(f)indazole. Tetra-n-butyl-ammonium dihydrogen phosphate (0.28 mM.) was added to 3a,4,9,9a-

tetrahydro-9a-methyl-4,9-dioxo-3H-benz(f)indazole in DMSO (2 mls.). There was a vigorous evolution of nitrogen and the solution turned black. Examination of a sample of the resulting solution by paper chromatography (solvents B and C) showed inorganic phosphate as the only phosphorus-containing compound. The solution was diluted with water and the organic material filtered off (35.4 mgm., 77%) and recrystallised from dioxan to yield 10.8 mgm. (23.5% yield) of a compound characterized as 3,3'-dimethyl-2,2'-ethylene-di-1,4-naphthoquinone (M.Pt. and mixed M.Pt. 268 - 270°C. IR and NMR spectra identical with authentic compound). Examination of the crude organic material by TLC showed the dimer (R_f= 0.192), an orange compound (R_f = 0.177) and much material remaining of the origin (R_f = 0). The experiment was repeated using di-tetra-n-butyl-ammonium monohydrogen phosphate and tri-tetra-n-butyl-ammonium phosphate with similar results. The three experiments were repeated using dioxan, dimethylformamide and acetonitrile as solvents with similar results. Variation of the relative quantity of phosphate was without effect.

(D) The Attempted Addition of Monophenyl Phosphate to the Quinone Methide Produced in Situ from 2,6-Di-t-Butyl-4-Bromo-4-Methyl-2,5-Cyclohexadien-1-One.

(i) Reaction of 2,6-Di-t-Butyl-4-Bromo-4-Methyl-2,5-Cyclohexadien-1-One with Methanol. A solution of the cyclohexadienone (203 mgm.) in

hot methanol (5 mls.) was heated under reflux with no apparent change. A few drops of methanol containing a trace of glacial acetic acid were added and the yellow colour of the solution rapidly disappeared. The solution was cooled and diluted with water and the organic material extracted with chloroform. The organic layer yielded on off white solid (172 mgm. 100% yield based on $C_{16}H_{26}O_2$).

ν (Nujol Mull)	3540 (med.broad); 1660 (v.st.d.)
τ (CCl_4)	2.92 (s.), 3.6 (s.), 4.96 (s.), 5.72 (s.), 6.7 (s.), 6.94 (s.), 8.56 (s.), 8.7 (s.), 8.78 (s.).

The crude mixture was recrystallised twice from methanol to yield, 2,6-di-t-butyl-4-methoxy-4-methyl-2,5-cyclohexadien-1-one (64% yield. Estimated from NMR spectrum of mixture).

M.Pt.	93 - 94°C (lit. (102) 94°C)
ν (Nujol Mull)	1660, 1640 (v.st.d. (179)) cm^{-1}.
τ (CCl_4)	3.6 (s.) 2H, 6.95 (s.), 3H, 8.68 (s.) 3H, 8.77 (s.) 18H.

The mother liquors were evaporated to dryness and the residue was recrystallised twice from petroleum ether (60 - 80°C) to yield 3,5-di-t-butyl-4-hydroxybenzyl methyl ether. (36% yield. Estimated from NMR spectrum of mixture).

M.Pt. and Mixed M.Pt.	98 - 99°C (lit. (102) 99.5°C)
ν (Nujol Mull)	3540 (v.st. broad) cm^{-1}
τ (CCl_4)	2.94 (s.) 2H, 4.98 (s.) 1H, 5.72 (s.) 2H, 6.7 (s.) 3H, 8.56 (s.) 18H.

(ii) The Reaction of 2,6-Di-t-Butyl-4-Bromo-4-Methyl-2,5-Cyclohexadien-1-One with Disilver Monophenyl Phosphate. Disilver monophenyl phosphate (1.15 mM.) was added to the cyclohexadienone (1 mM.) in dry acetonitrile (4 mls.) and the mixture shaken overnight at room temperature. The precipitate was filtered off and proved to be silver bromide by qualitative tests. The resulting solution was examined directly by NMR spectroscopy but showed no characteristic doublet in the region 4 - 6.9 τ due to the spin-spin coupling of the hydrogens of a benzyl group with a phosphorus atom. Examination of the solution by paper chromatography (solvents B and C) showed monophenyl phosphate as the only phosphorus-containing compound. Examination by TLC (elution with benzene) showed several products (R_f = 0.5 yellow, R_f = 0.095 dark yellow, R_f = 0 brown). The bands were broad and separation was incomplete. The compounds could not be completely identified but enolic and carbonyl compounds were shown to be present.

THE ATTEMPTED SYNTHESIS AND OXIDATION OF SOME 4-HYDROXYBENZYL ESTER SYSTEMS

THE ATTEMPTED SYNTHESIS AND OXIDATION OF SOME 4-HYDROXYBENZYL ESTER SYSTEMS

(1) Bis-2, 2, 2-Trichloroethyl Phosphorochloridate (155) was prepared by a method analogous to that described for 2, 2, 2-trifluoroethyl phosphorochloridate in Houben Weyl (180). A solution of pyridine (55.6 gm.) and 2, 2, 2-trichloroethanol (105.5 gm.) in ether (50 mls.) was added dropwise to a solution of phosphorus oxychloride (54 gm.) in ether (80 mls.), cooled in ice. After 30 minutes at room temperature the precipitate of pyridinium chloride was filtered off and washed well with ether. The filtrate was fractionally distilled under vacuum.

B.Pt. $138^{o}C/1$ mm. Hg. Yield 77 gm. (58%)

τ (CCl_4) 5.2 (d., $J_{P-H} = 8$ c/s).

(2) 4-Methoxymethoxybenzaldehyde (181) was prepared by a method analogous to that described for 2-nitro-4, 5-bis-methoxymethoxy-benzaldehyde (182). 4-Hydroxy-benzaldehyde (25.5 gm.) was added to sodium methoxide (11.3 gm.) in methanol (200 mls.). The solution was evaporated to dryness and the sodium salt obtained was dried in a vacuum oven and ground to a fine powder. A suspension of this salt in benzene (200 mls.) containing chloromethyl ether (16.8 gm.) was heated under reflux for two hours. The mixture was cooled and the

precipitate of sodium chloride was filtered off. The benzene solution was washed with dilute aqueous caustic soda solution and then with water. After drying the benzene solution over magnesium sulphate and removing the benzene, 4-methoxymethoxy-benzaldehyde (21.5 gm. 62% yield) was obtained as a pale yellow oil.

ν (Thin film)	1690 (v.st.), 1150 (v.st.) cm^{-1}
τ (CCl_4)	0.13 (s.) 1H, 2.56 (ABq., $\Delta\nu_{AB}$ = 39.0 c/s., J = 9.0 c/s.) 4H, 4.8 (s.) 2H, 6.55 (s.) 3H.

Analysis of semicarbazone :

Found	C, 53.95 ; H, 5.87 ; N, 18.95%
Calc. for $C_{10}H_{13}O_3N_3$:	C, 53.79 ; H, 5.87 ; N, 18.82%

(3) 4-Methoxymethoxybenzyl Alcohol (183). Soldium borohydride (4 gm.) in a minimum of dilute aqueous caustic soda was added slowly to 4-methoxymethoxy-benzaldehyde (21.5 gm.) in methanol (200 mls.). After a few minutes at room temperature the solution was neutralised with dilute hydrochloric acid (phenolphthalein as external indicator) and the methanol removed by distillation under reduced pressure. The residue was extracted with ether and the ethereal extract, after being washed with water, was dried over magnesium sulphate and gave 4-methoxymethoxybenzyl alcohol (21 gm. 97% yield) as a pale yellow oil.

ν (Thin Film)	3380 (v.st. broad), 1150 (v.st.) cm^{-1}
τ (CCl_4)	3.05 (ABq., $\Delta\nu_{AB}$ = 13.1 c/s., J = 8.4 c/s.) 4H, 5.02 (s.) 2H, 5.7 (s.) 2H, 6.05 (s. broad) 1H, 6.7 (s.) 3H.

Analysis of 3,5-di-p-nitrobenzoate :

Found	C, 53.21 ; H, 3.96 ; N, 7.80%
Calc. for $C_{10}H_{13}O_3N_3$:	C, 53.04 ; H, 3.87 ; N, 7.74%

(4) 4-Methoxymethoxybenzyl Benzoate. Benzoyl chloride (1.68 gm.) was added dropwise to 4-methoxymethoxybenzyl alcohol (2 gm.) in pyridine (15 mls.) and the mixture left overnight at room temperature. The mixture was then diluted with water and extracted with ether. The ethereal extract was dried over magnesium sulphate after several washings with water, and the ether removed by distillation. Last traces of pyridine were removed by freeze drying the crude product. 4-methoxymethoxybenzyl benzoate (3.11 gm. 96% yield) was obtained as a pale yellow oil.

ν (Thin Film)	1720 (v.st.), 1150 (st.) cm^{-1}
τ (CCl_4)	1.75 - 3.05 (complex m.) 9H, 4.75 (s.) 2H, 4.89 (s.) 2H, 6.20 (s.) 3H.

Analysis :

Found	C, 70.13 ; H, 5.92%
$C_{16}H_{16}O_4$ requires	C, 70.55 ; H, 5.93%

(5) Bis-2, 2, 2-Trichloroethyl 4-Methoxymethoxybenzyl Phosphate

Bis-2, 2, 2-trichloroethyl phosphorochloridate (3.47 gm.) in ether (30 mls.) was added to 4-methoxymethoxybenzyl alcohol (1.55 gm.) in ether (20 mls.) containing pyridine (0.8 mls.) and the mixture was left overnight at room temperature. The precipitate of pyridinium chloride was filtered off and washed with ether. The filtrate, on removal of the ether, yielded bis-2, 2, 2-trichloroethyl 4-methoxy-methoxybenzyl phosphate (4.83 gm. 102% yield) as a colourless oil. An attempted purification resulted in decomposition of the ester.

ν (Thin Film)	1285 (st.), 1230 (s.), 1000 (v.st.) cm^{-1}
τ (CCl_4)	2.94 (ABq., $\Delta\nu_{AB}$ = 19.5 c/s., J = 7.8 c/s) 4H, 4.99 (d., J_{P-H} = 9.6 c/s.) 2H, 5.0 (s.) 2H, 5.58 (d., J_{P-H} = 6.6 c/s) 4H, 6.7 (s.) 3H

(6) Bis-2, 2, 2-Trichloroethyl Methyl Phosphate . Bis-2, 2, 2-trichloroethyl phosphorochloridate (3.04 gm.) was added to methanol (0.325 mls.) and pyridine (0.65 mls.) in benzene (50 mls.) and the mixture was left overnight at room temperature. The pyridinium chloride was filtered off and the filtrate, on removal of the benzene, gave bis-2, 2, 2-trichloroethyl

methyl phosphate (3.1 gm. 103% yield) as a waxy solid.

τ (CCl_4)	5.39 (d., J_{P-H} = 7.2 c/s) 4H,
	6.1 (d., J_{P-H} = 12 c/s) 3H

(7) Cleavage of 2, 2, 2-Trichloroethyl Groups from Bis-2, 2, 2-Trichloroethyl Methyl Phosphate. Excess zinc dust was added to bis-2, 2, 2-trichloroethyl methyl phosphate (200 mgm.) dissolved in (9/1) pyridine/water (5 mls.). The mixture was heated under reflux for 1 hour, cooled, and filtered through a strong cation exchange resin (Dowex 50W). Excess cyclohexylamine was added, followed by excess acetone. The white precipitate was filtered off and examined by paper chromatography (solvent C). This showed the following components were present :

R_f = 0	Trace P_i (yellow spot)
R_f = 0.16	Methyl Phosphate (intense blue spot) Major Product
R_f = 0.42	Trace unidentified phosphate monoester (blue spot). (See below)

(8) Cleavage of 2, 2, 2-Trichloroethyl Groups (154, 155) from Bis-2, 2, 2-Trichloroethyl 4-Methoxymethoxybenzyl Phosphate. Excess zinc dust was stirred overnight at room temperature with bis-2, 2, 2-trichloroethyl-4-methoxymethoxybenzyl phosphate (5.7 gm.) in 4/1, acetic acid/water (100 mls.). The resultant mixture was diluted with water and shaken

with an excess of a weak cation exchange resin (IRC50) and filtered. The filtrate was neutralised with aqueous lithium hydroxide solution and evaporated to dryness. The residue was washed with acetone until the washings gave no precipitate on addition of aqueous silver nitrate solution. Examination of the residue by paper chromatography (solvent C) showed inorganic phosphate ($R_f = 0$) and a phosphate monoester ($R_f = 0.42$) to be present. Purification of the residue by ion exchange chromatography through a column of Dowex 1 resin (100 - 200 mesh, 8% cross linking, flow rate 2 ml./minute, gradient elution of 0.0 - 0.25 M aqueous lithium chloride solution) yielded the dilithium salt of a phosphate monoester (81 mgm. 3% approx). This was characterised as dilithium 2, 2-dichloroethyl phosphate.

M.Pt.	$>360^{o}C$
τ (D_2O)	3.88 (t., J_{H-H} = 6 c/s.) 1H,
	4.80 (d. of d's. J_{H-H} = 6 c/s., J_{P-H} = 8.4 c/s) 2H
Analysis :	
Found	C, 11.32 ; H, 1.49 ; Cl, 32.88 ; P, 14.59%
$C_2H_3Cl_2Li_2O_4P$ requires	C, 11.38 ; H, 1.42 ; Cl, 33.65 ; P, 14.69%

Cleavage of the 2, 2, 2-trichloroethyl groups using a Zn/Cu coupled in DMF at $60^{o}C$, Zn dust in dioxan/.880 ammonia, zinc dust in pyridine/water (9/1) under reflux or 2, 6-lutidine/water (9/1) under reflux gave similar results. VPC analysis of the original 2, 2, 2-trichloroethanol

showed it to contain 3% of the 2, 2-dichloroethanol.

(9) Dicyclohexylammonium 4-Methoxymethoxybenzyl Phosphate

was prepared by oxidising phosphorous acid with iodine in the presence of the parent alcohol (158). Solid iodine (4 gm.) was added to a cooled solution of phosphorous acid (1 gm.) and triethylamine (5.5 mls.) in 4-methoxymethoxybenzyl alcohol (10 mls.). After the initial exothermic reaction was over, the mixture was poured into acetone (150 mls.) containing cyclohexylamine (12 mls.). The white crystals produced were filtered off and recrystallised from ethanol to yield dicyclohexylammonium 4-methoxymethoxybenzyl phosphate (2.9 gm., 53% yield). A sample of the salt was purified by ion-exchange chromatography through a column of Dowex 1 resin using gradient elution (0.0 - 0.25 M aqueous lithium chloride solution) at a flow rate of 2 ml./minute. The fractions containing the phosphate monoester were evaporated to dryness and the lithium chloride removed by repeated washing with acetone. Pure white crystals of dilithium 4-methoxymethoxybenzyl phosphate remained. Paper chromatography (solvent C) showed single spot (R_f = 0.325) which was yellow in colour and appeared slowly (i.e. decomposed to P_i by phosphate spray).

M.Pt. $>360^{\circ}C$

τ (D_2O)	2.75 (ABq., $\Delta\nu_{AB}$ = 19.7 c/s., J = 7.8 c/s.) 4H, 4.7 (s.), 5.16 (presumably half of doublet benzyl peak, the rest obscurred by water peak), 6.5 (s.) 3H.
Analysis :	
Found	C, 41.50 ; H, 4.10 ; P, 12.19%
$C_qH_{11}Li_2O_6P$ requires	C, 41.54 ; H, 4.23 ; P, 11.92%

(10) 4-Trimethylsiloxybenzaldehyde. N-trimethylsilyl acetamide (5.22 gm.) was added to 4-hydroxybenzaldehyde (4.88 gm.) in carbon tetrachloride (50 mls.) and the solution left overnight at room temperature. The precipitate of acetamide was filtered off and the carbon tetrachloride removed from the filtrate by distillation to give a residual oil of 4-trimethylsilylbenzaldehyde (7.8 gm., 108% yield).

ν (Thin Film)	1690 (v.st.) cm^{-1}
τ (CCl_4)	0.0 (s.) 1H, 2.55 (ABq., $\Delta\nu_{AB}$ = 56 c/s, J = 9 c/s) 4H, 9.68 (s.) 9H.

(11) Reduction of 4-Trimethylsiloxybenzaldehyde. 10% Palladium on charcoal (100 mgm.) was added to 4-trimethylsiloxybenzaldehyde in acetone (25 mls.) and the mixture hydrogenated at one atmosphere pressure. After the theoretical quantity of hydrogen had been consumed

the catalyst was filtered off and the acetone removed on the rotary evaporator. A light brown solid remained (319 mgm.) which turned red on standing. The NMR spectrum of the solid indicated a strong aldehyde peak but the trimethylsilyl peak was now weak. Thus the silyl ether was cleaved preferentially. The use of 10% Palladium on barium sulphate, Palladium black or Adam's catalyst as catalyst, or dry acetic acid as solvent gave similar results. The use of sodium borohydride as reducing agent resulted in immediate cleavage of the trimethylsilyl group.

(12) 4-Acetoxybenzaldehyde (184). Acetyl chloride (8 mls.) was added dropwise to a solution of 4-hydroxybenzaldehyde (12 gm.) in pyridine (20 mls.), cooled in ice. The mixture was left overnight at room temperature and poured into water, acidified with dilute hydrochloric acid and extracted with ether. The ethereal extract was dried over magnesium sulphate and, on removal of the ether, yielded 4-acetoxybenzaldehyde (13.8 gm., 84% yield) as a pale yellow oil.

ν (Thin film)	1760 (v.st.), 1700 (v.st.) cm^{-1}
τ (CCl_4)	0.0 (s.) 1H, 2.43 (ABq., $\Delta\nu_{AB}$ = 37.6 c/s, J = 8 c/s.) 4H, 7.75 (s.) 3H.

(13) 4-Acetoxybenzyl Alcohol (185). 10% Palladium on charcoal (2 gm.) was added to 4-acetoxybenzaldehyde (13.5 gm.) in acetone (125 mls. approx.) and the mixture hydrogenated at one atmosphere pressure (186). After the theoretical volume of hydrogen (1940 mls.) had been consumed the catalyst was filtered off and the acetone removed on the rotary evaporator. The residue was dissolved in chloroform and washed with aqueous sodium bisulphite solution followed by water. The dried chloroform extract yielded p-acetoxybenzyl alcohol (10 gm., 74% yield) as a pale yellow oil.

ν (Thin Film)	3420 (st. v. broad), 1760 (v.st.) cm^{-1}
τ (CCl_4)	2.92 (ABq., $\Delta\nu_{AB}$ = 15.1 c/s., J = 10.8 c/s.) 4H, 5.6 (s.) 2H, 6.44 (broad s.) 1H, 7.86 (s.) 3H.

Analysis of 3,5 dinitrobenzoate (M.Pt. 118 - 119°C)

Found	C, 53.47 ; H, 3.48 ; N, 7.65%
Calc. for $C_{16}H_{12}O_8N_2$:	C, 53.34 ; H, 3.33 ; N, 7.78%

(14) Dicyclohexylammonium 4-Acetoxybenzyl Phosphate. Solid iodine (4 gm.) was added to a solution of phosphorous acid (1 gm.) in triethylamine (5.5 mls.) and p-acetoxybenzyl alcohol (9 gm.), cooled in ice. After the initial exothermic reaction was completed the mixture was poured into methyl ethyl ketone (150 mls.) and cyclohexylamine (12 mls.) added. The mixture was extracted with water and the aqueous

layer extracted several times with ether before it was evaporated to dryness and the residue recrystallised from ethanol to yield dicyclohexylammonium 4-acetoxybenzyl phosphate (2.51 gm. 48% yield). A sample was purified by the usual ion-exchange chromatography procedure to yield pure dilithium 4-acetoxybenzyl phosphate.

M.Pt.	>360°C
Paper Chromatography	Solvent B : R_f = 0.75, yellow spot appears slowly Solvent C : R_f = 0, Inorganic phosphate, i.e. unstable in this system.
τ (D_2O)	2.57 (ABq., $\Delta\nu_{AB}$ = 21.6 c/s., J = 9 c/s.) 4H, 5.04 (d., J_{P-H} = 7.8 c/s.). Too close to water peak for integration, 7.65 (s.) 3H
Analysis :	
Found	C, 41.71 ; H, 3.37 ; P, 11.71%
$C_qH_qLi_2O_6P$ requires	C, 41.86 ; H, 3.49 ; P, 12.02%

(15) 4-Hydroxy-3,5-Di-t-Butylbenzyl Piperidine-1-Carbodithiate was prepared by the reaction of paraformaldehyde, piperidine and carbon disulphide with 2,6-di-t-butylphenol (105).

M.Pt.	112 - 113°C (lit. (105) 112 - 113°C

(16) 4-Hydroxy-3,5-Di-t-Butylbenzyl Acetate was prepared by the decomposition of the above carbodithioate in glacial acetic acid containing nickel acetate (105).

M.Pt.	104 - 105°C (lit. (102) 98°C, lit. (105) 102°C)
ν (Nujol Mull)	3530 (v. st. broad), 1720 (v.st.) cm^{-1}
τ (CCl_4)	2.75 (s.) 2H, 4.96 (s.) 2H, 7.92 (s.) 3H, 8.58 (s.) 18H.

(17) Removal of the Protecting Group from Dicyclohexylammonium 4-Methoxymethoxybenzyl Phosphate. Dicyclohexylammonium 4-methoxymethoxybenzyl phosphate (100 mgm.) was dissolved in water (3 mls.) and Dowex 50W cation exchange resin (100 mgm. approx.) added. The mixture was heated under reflux for a few seconds, cooled and filtered. Examination of the filtrate by paper chromatography (solvents B and C) showed inorganic phosphate as the only phosphorus-containing compound. Thus, under these conditions, 4-hydroxybenzyl phosphate is unstable.

(18) Removal of Protecting Group from Dicyclohexylammonium 4-Acetoxybenzyl Phosphate. Dicyclohexylammonium 4-acetoxybenzyl phosphate (100 mgm.) was dissolved in water (3 mls.) and cyclohexylamine (3 drops) added. The mixture was heated under reflux for a few seconds, and cooled. Examination of the solution by paper chromatography (solvent B) showed inorganic phosphate as the only phosphorus-containing

compound. Thus, under these conditions also, 4-hydroxybenzyl phosphate is unstable.

(19) Attempted Observation of Oxidative Phosphorylation using a substrate, 4-Hydroxybenzyl Phosphate produced in Situ.

(i) Dicyclohexylammonium 4-Methoxymethoxybenzyl Phosphate (0.125 mM) was added to absolute ethanol (5 mls.) N-Bromsuccinimide (0.5 mM.) was added followed by Dowex 50W (50 mgm. approx.) and the mixture shaken. Samples were examined by paper chromatography (solvents B and C) at convenient intervals up to 24 hours. Inorganic phosphate and starting material were the only phosphorus-containing compounds observed. There was no trace of ethyl phosphate. The experiment was repeated using t-butyl hypochlorite (187), dicyano-dichloro-p-benzoquinone, thallium acetate/thallic oxide, and ceric ammonium nitrate as oxidising agents with similar results. All 5 experiments were repeated with the omission of Dowex 50W with similar results. All 10 experiments were repeated heating the mixtures under reflux for 5 minutes with similar results. The 20 experiments were repeated using dioxan in place of ethanol with similar results. No pyrophosphate was detected.

(ii) Dicyclohexylammonium 4-Acetoxybenzyl Phosphate (0.124 mM.) was added to absolute ethanol (5 mls.). N-Bromsuccinimide (0.5 mM.) was added followed by cyclohexylamine (3 drops) and the mixture warmed and then cooled. Examination of the mixture by paper chromatography (solvent B) showed inorganic phosphate as the only phosphorus-containing compound. The experiment was repeated using t-butyl hypochlorite, dicyano-dichloro-p-benzoquinone, thallous acetate/thallic oxide and ceric ammonium nitrate as oxidising agents with similar results. No ethyl phosphate was detected.

(20) The Decomposition of 4-Methoxymethoxybenzyl Benzoate in Acidic Methanol. 4-methoxymethoxybenzyl benzoate (515 mgm.) in methanol (25 mls.) containing concentrated sulphuric acid (4 drops) was heated under reflux for 4 minutes. Most of the methanol was removed on the rotary evaporator. The remaining mixture was diluted with IN aqueous caustic soda solution and extracted with ether. The ether extract was discarded, while the aqueous layer was acidified with dilute hydrochloric acid and extracted three times with ether. This ethereal extract was extracted twice with aqueous sodium bicarbonate solution and then dried over magnesium sulphate (ethereal extract (i)). The sodium bicarbonate extract was acidified with dilute hydrochloric acid and extracted twice with ether. This ethereal extract (ii) was also dried

over magnesium sulphate.

The ethereal extract (ii) yielded Benzoic Acid (233 mgm. 99% yield)

M.Pt. and mixed M.Pt.	121°C
ν (Nujol Mull)	identical with authentic compound

The ethereal extract (i) yielded 4-hydroxybenzyl methyl ether (261 mgm. 92% yield) as a pale yellow oil.

ν (Nujol Mull)	3300 (st.v. broad) cm^{-1}
τ ($CDCl_3$)	3.12 (ABq., $\Delta\nu_{AB}$ = 25.4 c/s., J = 9 c/s.) 4H, 5.68 (s.) 2H, 6.7 (s.) 3H.

The phenol in methylcyclohexane (25 mls.) was heated under reflux with 1-naphthylisocyanate for ½ hour and then left standing for 3 days at room temperature. The 1-naphthylisocyanate derivative was filtered off and recrystallised from methylcyclohexane.

M.Pt.	113 - 115°C
ν (Nujol Mull)	3240 (st. broad), 1700 (v.st.) $cm.^{-1}$
τ ($CDCl_3$)	1.97 - 2.7 (complex m.) 12H, 5.53 (s.) 2H, 6.6 (s.) 3H.
Analysis	Parent peak in mass spectrometer at m/e = 307.120 corresponding to $C_{19}H_{17}NO_3$ as required.

(21) Attempted Observation of Oxidative Benzoylation Using as Substrate, 4-Hydroxybenzyl Benzoate Produced in Situ. N-Bromsuccinimide (40 mgm.), followed by Dowex 50W resin (50 mgm. approx.), was added to 4-methoxymethoxybenzyl benzoate (30 mgm.) in 50% ethanolic chloroform (1 ml.). The mixture was shaken and samples were examined for ethyl benzoate by VPC at convenient intervals up to 2 hours. The mixture was boiled and after cooling again examined for ethyl benzoate by VPC. In no case was ethyl benzoate detected. The experiment was repeated using t-butyl hypochlorite, dicyano-dichloro-p-benzoquinone, thallous acetate/thallic oxide, ceric ammonium nitrate as oxidising agents with similar results. The five experiments were repeated with the omission of Dowex 50W with similar results. All experiments were repeated with benzyl benzoate in place of 4-methoxymethoxybenzyl benzoate with similar results.

Instruments	Perkin Elmer F11. Flame Ionisation detector.
Column	20% L.A.C. - 2 R-446 on chromosorb P. HMDS treated. 2' x 1/8". Oven temperature 90°C. Injection Temp. Setting 5.5
Gas Pressures	H_2, 26 ; N_2, 15 ; air 19 lbs./sq.in.
Sample	0.6 µl.

Ethyl benzoate (1% solution in chloroform/ethanol (1/1)) had a retention time of 4 minutes and could be detected in 0.01% solution.

(22) Attempted Observation of Oxidative Acylation Using 2,6-Di-t-Butyl-4-Hydroxybenzyl Acetate as Substrate. N-Bromsuccinimide (40 mgm.) was added to 2,6-di-t-butyl-4-hydroxybenzyl acetate (20 mgm.) in 50% ethanolic chloroform (1 ml.). The mixture was shaken and examined by VPC for ethyl acetate at convenient intervals up to 1 hour. The mixture was heated under reflux for 5 minutes, cooled and examined by VPC for ethyl acetate again. No ethyl acetate was ever observed. The experiment was repeated using t-butyl hypochlorite, dicyano-dichloro-p-benzoquinone, thallous acetate/thallic oxide and ceric ammonium nitrate as oxidising agents with similar results. The analytical set up was the same as above except that a capilliary column was used and the oven was at room temperature. Ethyl acetate (1% solution in ethanol/chloroform (1/1)) had a retention time of 96 seconds and could be detected in 0.01% solution.

REFERENCES

REFERENCES

1. F.L. Crane and H. Low, Physiol. Rev., 1966, 46, 662.

2. E. Lederer and M. Vilkas, Vitamins and Hormones, 1966, 24, 409.

3. G. Schatz, Angew. Chem. Internat. Edn., 1967, 6, 1035.

4. V.M. Clark and D.W. Hutchinson, Progr. Org. Chem., 1968, 7, 75.

5. F.L. Crane, Y. Hatefi, R.L. Lester, and C. Widmer, Biochim. Biophys. Acta, 1957, 25, 220.

6. F.L. Crane, C. Widmer, R.L. Lester, and Y. Hatefi, Biochim. Biophys. Acta, 1959, 31, 476.

7. J.A. Anderson, Biochim. Biophys. Acta, 1964, 89, 540.

8. L. Szarkowska, Arch. Biochem. Biophys., 1966, 113, 519.

9. E.R. Redfearn and J. Friend, Biochem. J., 1962, 84, 34P.

10. M.M. Weber and G. Rosso, Proc. Nat. Acad. Sci. U.S.A., 1963, 50, 710.

11. A.F. Brodie and J. Ballantine, J. Biol. Chem., 1960, 235, 226, 232.

12. R.J. Downey, J. Bact., 1964, 88, 904.

13. W.P. Segel and D.S. Goldman, Biochim. Biophys. Acta, 1963, 73, 380.

14. A.F. Brodie, Science, 1967, 158, 1469.

15. S.J. DiMari and H. Rapoport, Biochemistry, 1968, 7, 2650.

16. C. Martius, H. Rieling, and D. Nitz-Litzow, Biochem.Z., 1955, 327, 163.

17. R.G. Wyn Jones, Biochem. J., 1967, 103, 714.

18. G.B. Cox, A.M. Snoswell, and F. Gibson, Biochim. Biophys. Acta, 1968, 153, 1.

19. Y. Hatefi, Biochim. Biophys. Acta, 1959, 31, 502.

20. E.R. Redfearn and A.M. Pumphrey, Biochem. Biophys. Res. Comm., 1960, 6, 50.

21. L. Szarkowska and M. Klingenberg, Biochem. Z., 1963, 338, 674.

22. F.L. Crane, Y. Hatefi, R.L. Lester, and L. Widmer, Biochim. Biophys. Acta, 1957, 25, 439.

23. A.M. Pumphrey, E.R. Redfearn, and R.A. Morton, Chem. and Ind., 1958, 978.

24. B. Chance, "Biochemistry of Quinones" (R.A. Morton, Ed.), Academic Press, N.Y., 1965, p.459.

25. A. Kroger and M. Klingenberg, Biochem. Z., 1966, 344, 317.

26. E. Racker, "Mechanisms in Bioenergetics", Academic Press, N.Y., 1965.

27. E.C. Slater, Nature, 1953, 172, 975.

28. E. Racker, J. Biol. Chem., 1951, 190, 685.

29. F. Lipmann, "Currents in Biochemical Research" (D.E. Green, Ed.), Interscience, N.Y., 1946.

30. W.F. Loomis and F. Lipmann, J. Biol. Chem., 1948, 173, 807.

31. H.A. Lardy, D. Johnson, and W.L. McMurray, Arch. Biochem. Biophys., 1958, 78, 587.

32. H.A. Lardy and W.L. McMurray, Fed. Proc., 1959, 18, 269.

33. P.D. Boyer, W.W. Luchsinger, and A.B. Falcone, J. Biol. Chem., 1956, 223, 405.

34. M.A. Swanson, Biochim. Biophys. Acta, 1956, 20, 85.

35. I.A. Rose and S. Ochoa, J. Biol. Chem., 1956, 220, 307.

36. C.L. Wadkins and A.L. Lehninger, J. Biol. Chem., 1963, 238, 2555.

37. M. Cohn, J . Biol. Chem., 1953, 201, 735.

38. I. Vallin and H. Loew, Biochim. Biophys. Acta, 1964, 92, 446.

39. L. Danielson and L. Ernster, Biochem. Z., 1963, 338, 188.

40. L. Ernster in "Proceedings of the 5th International Congess of Biochemistry. Moscow 1961", I.U.B Symposium Series, Pergamon Press, N.Y., 1963, 25, 115.

41. J.M. Tager and E.C. Slater, Biochim. Biophys. Acta, 1963, 77, 227.

42. J.S.C. Wessels, Rec. Trav. Chim., 1954, 73, 529.

43. C. Martius, Biochem. Z., 1956, 347, 407.

44. D.I. Arnon, M.B. Allen, and F.R. Whatley, Biochim. Biophys. Acta, 1956, 20, 449.

45. R.D. Dallam and W.W. Anderson, Biochim. Biophys. Acta, 1957, 25, 439.

46. A.F. Brodie, M.M. Weber, and C.T. Gray, Biochim. Biophys. Acta, 1957, 25, 448.

47. J. Bouman and E.C. Slater, Biochim. Biophys. Acta, 1957, 26, 624.

48. K. Harrison, Nature, 1958, 181, 1131.

49. R.E. Beyer, J. Biol. Chem., 1959, 234, 398.

50. T. Wieland and F. Pattermann, Chem. Ber., 1959, 92, 2917.

51. R.F. Hudson, "Structure and Mechanism in Organo-Phosphorus Chemistry", Academic Press, 1965, p.58.

52. V.M. Clark, D.W. Hutchinson, A.J. Kirby, and S.G. Warren, Angew. Chem., 1964, 76, 704.

53. L. Larsson, Svensk Kem. Tidskr., 1958, 70, 405.

54. T. Wieland and F. Pattermann, Angew. Chem., 1958, 70, 313.

55. V.M. Clark, ~~D.W. Hutchinson~~, G.W. Kirby, and Sir Alexander Todd, Nature, 1958, 181, 1650.

56. V.M. Clark, D.W. Hutchinson, G.W. Kirby, and Sir Alexander Todd, J. Chem. Soc., 1961, 715.

57. V.M. Clark, D.W. Hutchinson, and Sir Alexander Todd, Nature, 1960, 187, 59.

58. V.M. Clark, D.W. Hutchinson, and Sir Alexander Todd, J. Chem. Soc., 1961, 722.

59. V.M. Clark, "14th Mosbacher Colloqium", Springer Verlag., 1964, p.276.

60. P.D. Boyer, "Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto, 1957", Manzen, Tokyo, 1958, p.301.

61. T. Wieland and F. Pattermann, Angew. Chem., 1958, 70, 313.

62. A. Todd, Proc. Nat. Acad. Sci. U.S.A., 1959, 45, 1389.

63. R.D. Dallam and L.H. Chen, Arch. Biochem. Biophys., 1968, 123, 565.

64. A. Lapidot and D. Samuel, J. Amer. Chem. Soc., 1964, 86, 1886.

65. A. Lapidot and D. Samuel, Biochim. Biophys. Acta, 1962, 65, 164.

66. W. Duerckheimer and L.A. Cohen, Biochemistry, 1964, 3, 1948.

67. G.E. Tomasi and R.D. Dallam, Fed. Proc., 1962, 21, 53.

68. G.E. Tomasi and R.D. Dallam, J. Biol. Chem., 1964, 239, 1604.

69. J.S. Cohen and A. Lapidot, J. Chem. Soc. (C), 1967, 1210.

70. H.C. Khorana and A.R. Todd, J. Chem. Soc., 1953, 2257.

71. F. Cramer, Chem. and Ind., 1960, 46.

72. F. Cramer and H. Winter, Chem. Ber., 1959, 92, 2761.

73. J. Wodak, J. Amer. Chem. Soc., 1968, 90, 2991.

74. V.M. Clark and Sir Alexander Todd, "Quinones in Electron Transport" (Wolstenholme and O'Connor, Eds), Churchill, London, 1961, p.190.

75. C.D. Snyder and H. Rapoport, Biochemistry, 1968, 7, 2318.

76. R.C. Fuson, Chem. Revs., 1935, 16, 1.

77. I. Chmielewska, Biochim. Biophys. Acta, 1960, 39, 170.

78. M. Vilkas and E. Lederer, Experientia, 1963, 18, 546.

79. A.F. Brodie, "Biochemistry of Quinones" (R.A. Morton, Ed.), Academic Press, London, 1965, p.386.

80. P.J. Russell and A.F. Brodie, Biochim. Biophys. Acta, 1961, 50, 76.

81. G.E. Horth, D. McHale, I.R. Jeffries, S.A. Price, A.T. Diplock, and J. Green, Biochem. J., 1966, 100, 424.

82. C.D. Snyder, S.J. DiMari, and H. Rapoport, J. Amer. Chem. Soc., 1966, 88, 3868.

83. W.W.Parson and H. Rudney, Biochemistry, 1966, 5, 1013.

84. D.L. Gutnick and A.F. Brodie, J. Biol. Chem., 1965, 240, 3698.

85. F. Scherrer, R. Azerad, and M. Vilkas, Experientia, 1967, 23, 360.

86. S.J. DiMari, C.D. Snyder, and H. Rapoport, Biochemistry, 1968, 7, 2301.

87. P.M. Scott, J. Biol. Chem., 1965, 240, 1324.

88. A. Lapidot, B.L. Silver, and D. Samuel, Biochem. Biophys. Res. Comm., 1965, 21, 126.

89. A. Lapidot, B.L. Silver, and D. Samuel, J. Biol. Chem., 1966, 241, 5537.

90. D.W. Cameron, P.M. Scott, and Lord Todd, J. Chem. Soc., 1964, 42.

91. A.F. Wagner, A. Lusi, C.H. Shunk, B.O. Linn, D.E. Wolf, C.H. Hoffmann, R.E. Erickson, B. Arison, R.N. Trenner, and K. Folkers, J. Amer. Chem. Soc., 1963, 85, 1534.

92. P. Mamont, P. Cohen, R. Azerad, and M. Vilkas, Bull. Soc. Chim. France, 1965, 2513.

93. P. Mamont, P. Cohen, R. Azerad, and M. Vilkas, Bull. Soc. Chim. France, 1965, 2824.

94. L.I. Smith, R. Tess, and G.E. Ullyot, J. Amer. Chem. Soc., 1944, 66, 1320.

95. K.H. Meyer, Annalen, 1920, 420, 134.

96. R.W. Martin, "The Chemistry of Phenolic Resins", Wiley, N.Y., 1956, p.139.

97. A.B. Turner, Quart. Rev., 1964, 18, 347.

98. E.R. Altwicker, Chem. Rev., 1965, 67, 475.

99. C.D. Cook and D.E. Norcross, J. Amer. Chem. Soc., 1956, 78, 379.

100. A.N. Al-Khafaji, Dissertation Ph.D. Thesis, University of Texas, 1966.

101. H.D. Becker, J. Org. Chem., 1967, 32, 4093.

102. G.M. Coppinger and T.W. Campbell, J. Amer. Chem. Soc., 1953, 75, 734.

103. F.M. Dean, L.E. Houghton, and R.B. Morton, J. Chem. Soc. (C), 1967, 1980.

104. L.J. Filar and S. Winstein, Tet. Lett., 1960, 25, 9.

105. A.O. Fitton, A. Rigby, and R.J. Hurlock, J. Chem. Soc. (C), 1968, 996.

106. R.H. Bauer and G.M. Coppinger, Tetrahedron, 1963, 19, 1201.

107. R.B. Thompson, U.S.P., 3017, 422/1962.

108. M. Vilkas, M. Wakselman, and G. Decodts, Bull. Soc. Chim. France, in preparation 1966, see Ref. 2.

109. K.W. Rosenmund and F. Zetzsche, Ber., 1921, 54, 2038.

110. T. Watanabe and A.F. Brodie, Fed. Proc., 1966, 56, 940.

111. A.F. Wagner, P.E. Wittreich, B. Arison, N.R. Trenner and K. Folkers, J. Amer. Chem. Soc., 1963, 85, 1178.

112. A.F. Brodie, Fed. Proc., 1961, 20, 995.

113. A. Asano, A.F. Brodie, A.F. Wagner, P.E. Wittreich, and K. Folkers, J. Biol. Chem., 1962, 237, PC2411.

114. A. Asano, A.F. Brodie, A.F. Wagner, P.E. Wittreich, and K. Folkers, Fed. Proc., 1962, 21, 54.

115. W. Gruber, R. Hohl, and T. Wieland, Biochem. Biophys. Res. Comm., 1963, 12, 242.

116. K.J.M. Andrews, J. Chem. Soc., 1961, 1808.

117. A. Lapidot and J.S. Cohen, J. Biol. Chem., 1966, 241, 4060.

118. M. Young, Biochem. Biophys. Res. Comm., 1968, submitted.

119. R.E. Erickson, A.F. Wagner, and K. Folkers, J. Amer. Chem. Soc., 1963, 85, 1535.

120. R.E. Beyer, Arch. Biochem. Biophys., 1968, 125, 884.

121. T. Wieland and E. Baeuerlein, Monatsh., 1967, 98, 1381.

122. T. Wieland and E. Baeuerlein, Chem. Ber., 1967, 100, 3869.

123. T. Wieland and E. Baeuerlein, Naturwiss. 1967, 54, 80.

124. P. Mitchell, Nature, 1961, 191, 144.

125. J.B. Chappell and A.R. Crofts, Biochem. J., 1965, 95, 393.

126. P. Mitchell and J. Moyle, Biochem. J., 1967, 104, 588.

127. U. Hopfer, A.L. Lehninger, and T.E. Thompson, Proc. Nat. Acad. Sci. U.S.A., 1968, 59, 484.

128. P. Mitchell and J. Moyle, Nature, 1965, 208, 147.

129. P. Mitchell and J. Moyle, Nature, 1965, 208, 1205.

130. B. Chance and L. Mela, Nature, 1966, 212, 257.

131. R.S. Cockrell, E.J. Harris, and B.C. Pressman, Biochemistry, 1966, 5, 2326.

132. D.N. Kursanov and Z.N. Parnes, Doklady Akad. Nauk S.S.S.R., 1955, 103, 847.

133. D.N. Kursanov and V.W. Setkina, Doklady Akad. Nauk S.S.S.R., 1954, 94, 69.

134. W.D. Waters and K.F. Bonhoeffer, Z. Physik. Chem., 1938, A182, 265.

135. S.K. Hsue, C.K. Ingold, and C.C. Wilson, J. Chem. Soc., 1938, 78.

136. W.S. Benedict, K. Morikawa, R.B. Barnes, and H.S. Taylor, J. Chem. Phys., 1937, 5, 1.

137. V.P. Guinn, "Liquid Scintillation Counting" (Bell and Hayes, Eds.), Pergamon Press, N.Y., 1958, p.166.

138. D.F. Eggers, N.W. Gregory, G.D. Halsey, and B.S. Rabinovitch, "Physical Chemistry", John Wiley and Sons, Inc., N.Y., 1964, p. 435.

139. V. Gold, "pH Measurements", Methuen, London, 1956, p.104.

140. H.T.S. Britton and R.A. Robinson, J. Chem. Soc., 1931, 1456
1937, 1848.

141. J.H. Bowie, D.W. Cameron, and D.H. Williams, J. Amer. Chem. Soc., 1965, 87, 5094.

142. R.H. Bible, "Interpretation of NMR Spectra", Plenum Press, N.Y., 1965, p.61.

143. H. Simon and D. Palm, Angew.Chem., 1966, 78, 993.

144. K.B. Wiberg, Chem. Rev., 1955, 55, 713.

145. W.F.K. Wynne-Jones, Trans. Faraday Soc., 1936, 32, 1397.

146. W.E. Nelson and J.A.V. Butler, J. Chem. Soc., 1938, 957.

147. D. Martin, Angew. Chem. Internat. Edn., 1967, 6, 318.

148. Pamela Lilley, unpublished results.

149. D.J. Cram and G.S. Hammond, "Organic Chemistry", McGrawHill, N.Y., 1964, p. 253.

150. H.D. Becker, J. Org. Chem., 1965, 30, 982.

151. W.E. Conrad and S.M. Dec, J. Org. Chem., 1958, 23, 1700.

152. J.F.W. McOmie, Adv. Org. Chem., 1963, 3, 191.

153. R.B. Woodward, K. Heusler, J. Gosteli, P. Naegli, W. Oppol zer, R. Ramage, S. Ranganathan, and H. Vorbrueggen, J. Amer. Chem. Soc., 1966, 88, 852.

154. F. Eckstein, Angew. Chem. Internat. Edn., 1965, 4, 876.

155. F. Eckstein and K.H. Scheit, Angew. Chem., 1967, 79, 317.

156. V.M. Clark and A.R. Todd, J. Chem. Soc., 1950, 2030.

157. L. Zervas and I. Dilaris, J. Amer. Chem. Soc., 1955, 77, 5354.

158. A.J. Kirby, Chem. and Ind., 1963, 1877.

159. C.S. Hanes and F.A. Isherwood, Nature, 1949, 164, 1107.

160. L. Birkofer and A. Ritter, Angew. Chem. Internat. Edn., 1965, 4, 417.

161. E. Akerman, Acta Chem. Scand., 1956, 10, 298.

162. L.I. Smith, Org. Syntheses, 1943, Coll. Vol.2, 254.

163. H. von Pechmann, Ber., 1888, 21, 1420.

164. E.A. Braude and J.S. Fawcett, Org. Syntheses, 1963, Coll. Vol. 4, 698.

165. O. Kruber, Ber., 1929, 62, 3044.

166. L. Rugheimer and M. Hankel, 1896, 29, 2180. Ber.

167. K. Chandrasenan and R.H. Thompson, J. Chem. Soc., 1966, 123.

168. E. Bergmann and F. Bergmann, J. Org. Chem., 1939, 3, 125.

169. O. Isler, R. Rueegg, A. Studer, and R. Tuergens, Z. Physiol. Chem., 1953, 295, 290.

170. G.H. Stillson, D.W. Sawyer, and C.K. Hunt, J. Amer. Chem. Soc., 1945, 67, 303.

171. C.D. Cook and D.E. Norcross, J. Amer. Chem. Soc., 1959, 81, 1176.

172. I.C. Fries and W. Lohmann, Ber., 1921, 54, 2912.

173. A.I. Vogel, "A Textbook of Practical Organic Chemistry", Longmans, Green and Co., London, 1956, 3rd Edn., p.969.

174. F.M. Dean, P.G. Jones, R.B. Morton, and P. Sidisunthorn, J. Chem. Soc., 1963, 5336.

175. R.H. Thomson, J. Chem. Soc., 1953, 1196.

176. M.E. Foss and C.S. Gibson, J. Chem. Soc., 1949, 3075.

177. Canadian Ind. Ltd., B.P., 966 224/1964.

178. M.S. Kharasch and B.S. Joshi, J. Org. Chem., 1957, 22, 1435.

179. T.W. Campbell and G.M. Coppinger, J. Amer. Chem. Soc., 1952, 74, 1469.

180. K. Sasse, "Methoden der Organischen Chemie" (Houben-Weyl), Georg Thieme Verlag, Stuttgart, 1964, Vol. 2, p.276.

181. Hoering and Baum, D.R.P., 209608/1909.

182. J. Harley-Mason, J. Chem. Soc., 1948, 1244.

183. H. Bohnsack and W. Seibert, Reichstoffe Aromen Koeperpflegemittel, 1965, 15, 321.

184. F. Tiemann and H. Herzfeld, Ber., 1877, 10, 64.

185. R. Grice and L.N. Owen, J. Chem. Soc., 1963, 1947.

186. K.W. Rosenmund and F. Zetzsche, Ber., 1921, 54, 638.

187. F.J. Wolf and J. Weijlard, Org. Syntheses, 1963, Coll. Vol. 4, 125.

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Hydrogen Isotope Exchange in Methyl-quinones

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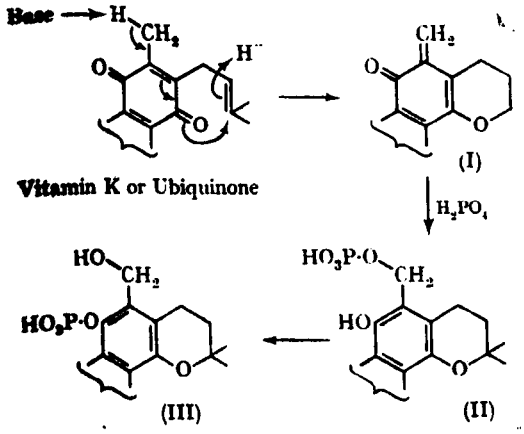
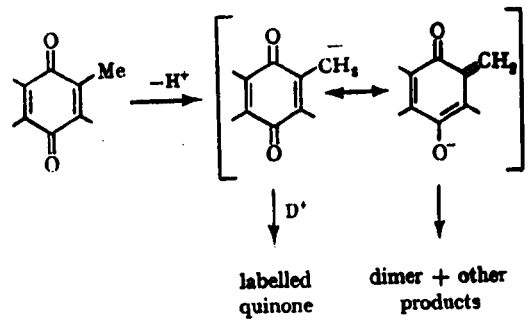
PHOSPHORYL TRANSFER accompanies the oxidation of hydroquinone phosphates.[1] This observation led to the suggestion[2] that the hydroquinone phosphates derived from vitamin K and ubiquinone might participate in the oxidative phosphorylation associated with electron transport in bacteria and mitochondria. Both these quinones contain a number of structural features in common,[3] particular attention having been paid to reactions involving the methyl group attached to the quinonoid nucleus. Vilkas and Lederer[3] proposed the intermediate formation of the quinone methide (I) leading, *via* the chromanyl phosphate (II), to (III). Isotopic evidence in support of the



proton removal from methyl quinones, necessary for this scheme, has been singularly elusive, both *in vivo*[4] and *in vitro*.[5]

We now report the base-catalysed incorporation of both deuterium and tritium into methyl-quinones thereby providing evidence for intermediate anions of the quinone methide type:



Thus, duroquinone, recovered after having been heated under reflux for several hours in dioxan–D_2O with triethylamine, or potassium carbonate, was found to contain deuterium, the infrared spectrum containing bands attributable to C–D stretching at 2260, 2220, 2130 and 2060 cm.$^{-1}$.

Using tritiated water, it was possible to determine the degree of isotope incorporation. In a typical reaction using triethylamine and 2,3-dimethylnaphthaquinone, isotopic equilibrium (in which the recovered quinone contained the same proportion of tritium as did the water) was reached after 10 hr. under reflux in aqueous dioxan. Vitamin K was unstable under these conditions but tritium uptake into perhydro-vitamin K was readily observed.

Isotope incorporation is greatly dependent on the nature of the base, temperature, and apparent pH (*cf.* ref. 5). Moreover, both the rate and the products of reaction are extremely sensitive to change in the type of base. Using cyclic secondary amines, *e.g.*, piperidine,[6] or pyrrolidine no

isotopically substituted methylquinone could be recovered. A fast and complex reaction involving the amine supervened. In the case of pyrrolidine and duroquinone, reaction was complete within a few minutes at room temperature; radical formation was indicated by a broad intense e.s.r. signal. This and other features are under further investigation.

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[1] T. Wieland and F. Pattermann, *Chem. Ber.*, 1959, **92**, 2917; V. M. Clark, D. W. Hutchinson, G. W. Kirby, and Sir Alexander Todd, *J. Chem. Soc.*, 1961, 715, 722.
[2] M. Vilkas and E. Lederer, *Experientia*, 1962, **18**, 546; E. Lederer and M. Vilkas, *Vitamins and Hormones*, 1966, **24**, 409.
[3] V. M. Clark, "Mechanismen Enzymatischer Reaktionen," Springer Verlag, 1964, p. 276.
[4] W. W. Parson and H. Rudney, *Biochemistry*, 1966, **5**, 1013; C. E. Horth, D. McHale, L. R. Jeffries, S. A. Price, A. T. Diplock, and J. Green, *Biochem. J.*, 1966, **100**, 424; C. D. Snyder and H. Rapoport, *J. Amer. Chem. Soc.*, 1967, **89**, 1269.
[5] A. Lapidot, B. L. Silver, and D. Samuel, *J. Biol. Chem.*, 1966, **241**, 5537.
[6] D. W. Cameron, P. M. Scott, and Lord Todd, *J. Chem. Soc.*, 1964, 42.